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EVALUATING NONSTRUCTURAL CARBOHYDRATE VARIATION OF COOL-SEASON GRASSES BASED ON GENOTYPE, MANAGEMENT AND ENVIRONMENT

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
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By

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ABSTRACT OF THESIS

EVALUATING NONSTRUCTURAL CARBOHYDRATE VARIATION OF COOL-SEASON GRASSES BASED ON GENOTYPE, MANAGEMENT AND ENVIRONMENT

Understanding how nonstructural carbohydrates fluctuate in pastures and being able to quantify them is an essential component in successfully managing grazing animals that may require high or low nonstructural carbohydrate diets. The objectives of this study were 1) to evaluate the effects of genotype, management, and environment on water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC) in cool-season grass pastures in central Kentucky, and 2) to develop near-infrared reflectance spectroscopy (NIRS) equations to predict WSC and ESC in cool-season grasses. Ten cool-season grass cultivars consisting of Kentucky bluegrass, tall fescue, orchardgrass and perennial ryegrass were sampled in the morning and afternoon every two to four weeks during the growing season from the University of Kentucky Research Farm in Lexington, KY. Samples were immediately flash frozen after sampling, freeze dried, ground, and scanned into FOSS 6500 NIRS with ISIscan software. WSC and ESC were determined using colorimetric phenol-sulfuric acid assays on a subset of samples and served as the basis for NIRS calibration. NIRS equations for both WSC and ESC accurately predicted wet chemistry values between 2% and 20%. Significant species and diurnal effects were observed for WSC and ESC. WSC and ESC concentrations were typically highest in the afternoon and lowest in the morning. Perennial ryegrass was the highest WSC accumulating species, followed by tall fescue, KY bluegrass, and orchardgrass. The effect of nitrogen fertility on WSC and ESC was inconsistent among harvest dates and cultivars. In conclusion, WSC and ESC in cool-season grasses are dependent on an interaction of factors including genotype, management and environment.

KEYWORDS: water-soluble carbohydrates, ethanol-soluble carbohydrates, near infrared reflectance spectroscopy, diurnal variation, pasture, sugar

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter One

Review of Literature

1.1 Carbohydrate Chemistry and Classification

Plants are primarily composed of carbohydrates, lipids, proteins, nucleic acids, vitamins, and minerals (Raven et al., 2005). Carbohydrates are therefore necessary for plant maintenance and development, and serve as the substrates for many compounds needed for growth. They are the most abundant organic molecules in nature and are made up of carbon, hydrogen and oxygen (Raven et al., 2005).

Monosaccharides are monomers that serve as the building blocks for other carbohydrates. They are linked carbons atoms with hydrogen and oxygen atoms attached in the proportion of one carbon to two hydrogens to one oxygen, or $(CH_2O)n$, in which the *n* can range from three to seven carbons (Raven et al., 2005). A common monosaccharide is glucose, a six carbon structure. Monosaccharides are classified by their functional group. For example, monosaccharides that contain an aldehyde group are called aldoses, some of which are glucose, galactose and xylose (Hatfield et al., 2007), and monosaccharides that contain a ketone group are called ketoses, one example being fructose. Disaccharides are two monosaccharides linked together, and trisaccharides are three monosaccharides linked together. An important disaccharide in the plant is sucrose, which is comprised of one molecule of glucose and one molecule of fructose. Derived from excess triose phosphate, sucrose is used as a major form of carbohydrate translocation within plants, is a major carbohydrate reserve compound and the major end-product of photosynthesis during rapid growth (Taiz and Zeiger, 1991).

Oligosaccharides are short chains of monosaccharides, and polysaccharides are long chains of monosaccharides. Some common polysaccharides include starch, cellulose and fructan. Fructan can occur in short chains or long chains of fructose units joined to a sucrose moiety (Longland and Byrd, 2006). Fructan only occurs in temperate C_3, or cool-season, plants, however, starch is present in all forages (Chatterton et al., 1989).

Carbohydrates are categorized as either structural or nonstructural. Structural carbohydrates are polysaccharides that are components of the cell wall and provide structural support to the plant (Raven et al., 2005), such as pectin, glycogen, cellulose and hemicellulose. Nonstructural carbohydrates serve in intermediary metabolism, energy transport and energy storage in the plant (Moore and Hatfield, 1994). Intermediary metabolism is comprised of pathways that synthesize, degrade, and transform important metabolites as well as conserve energy (Koolman and Röhm, 1999). Nonstructural carbohydrates include simple sugars (glucose, fructose, and sucrose), fructan, and starch. Total nonstructural carbohydrates (TNC) is a term used to encompass these carbohydrates, and is commonly used as an indicator of forage quality (Jensen et al., 2014). Other terms used to describe subsets of nonstructural carbohydrates are water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC). Ethanol or ethanol-water mixtures primarily extract simple sugars (glucose, fructose and sucrose) whereas water extracts simple sugars and fructan. Some studies have shown that ethanol can extract some short chain fructans in addition to simple sugars. In a study by Pavis et al. (2001), 80% ethanol extracted fructose, glucose, sucrose and fructans with a low degree of polymerization (DP, or chain length). Therefore, it may be more accurate to say that the difference between ethanol- and water-soluble carbohydrates consists mostly of long chain fructans. This thesis focuses on quantification of WSC and ESC, and therefore starch is not included in the nonstructural carbohydrates studied.

1.2 Nonstructural Carbohydrate Accumulation

Nonstructural carbohydrate accumulation occurs when carbohydrates produced from photosynthesis exceed amounts needed for plant growth and development (Watts and Chatterton, 2004). Excess carbohydrates produced from photosynthesis are delegated as reserve carbohydrates, which are stored during the day and a portion are degraded at night by respiration to produce energy for growth and maintenance (Preiss and Levi, 1980). Starch is the reserve carbohydrate for the seed of cool-season grasses, and fructan serves as the reserve carbohydrate for the vegetative tissues of cool-season grasses. Fructan is translocated from the leaf vacuoles to stem vacuoles to be stored as reserves, therefore

cool-season grasses typically accumulate high concentrations of fructan in parts of the lower stem or stem base (Waite and Boyd, 1953; Longland and Byrd, 2006). Sprague and Sullivan (1950) demonstrated variation in parts of the plant that accumulate the most carbohydrate. For example, fructan accumulated to the highest concentrations (36.2% on a dry weight basis) in the lower half of the stubble (vegetative parts of the plant excluding the leaf blades and roots), and sucrose accumulated to the highest concentrations (8.9% and 8.4%, respectively) in the roots and upper two-thirds of the leaf blades (Sprague and Sullivan, 1950).

Under conditions optimal for fructan accumulation, warm-season grasses and legumes typically produce less TNC than cool-season grasses because they do not produce fructan. Instead, warm-season grasses and legumes use starch as their main carbohydrate reserve (Chatterton et al., 1989). Jensen et al. (2014) observed significantly less fructan, WSC, ESC, and TNC in warm-season grasses than in cool-season grasses. They did detect small positive values in warm-season grasses when testing for fructan, which they suggested could be detection of other raffinose series oligosaccharides or other non-fructan sucrosyloligosaccharides (Jensen et al., 2014). Under cool temperatures, warm-season grasses accumulate primarily ESC and starch, while cool-season grasses mostly accumulate ESC and fructan, or WSC (Jensen et al., 2014).

1.3 Factors Affecting Nonstructural Carbohydrate Accumulation in Forages

Diurnal and Seasonal Variation

Carbohydrates are used and produced throughout various stages of the plant's life cycle, resulting in a flux of concentrations both seasonally (Waite and Boyd, 1953; Pollock and Jones, 1979; Shewmaker et al., 2006) and diurnally (Lechtenburg et al., 1972; Fisher et al., 1999; Shewmaker et al., 2006). Carbohydrates are produced through photosynthesis during the day and utilized through respiration. At night, photosynthesis does not occur and respiration continues to utilize carbohydrates for maintenance and growth (Watts and Chatterton, 2004). The combination of these processes results in a daily cycle that usually causes nonstructural carbohydrate concentrations to be lowest in the morning and highest

in the afternoon. Lechtenburg et al. (1972) observed significant increases in afternoon ESC concentrations in tall fescue (*Festuca arundinacea* Schreb.) when compared with the morning. In this case, most of the diurnal variation was attributed to sucrose, with slight fluctuation in glucose and no fluctuation in fructose. Of the amount of sucrose gained from morning to evening, 37% of it was lost (either respired or translocated) between six pm and midnight. Sixty-three percent of the gained sucrose was utilized after midnight (Lechtenburg et al., 1972). Kagan et al. (2011) noted higher WSC concentrations in the afternoon than in the morning in orchardgrass (*Dactylis glomerata* L.) on five out of eight springtime harvest dates. Fisher et al. (1999) demonstrated animal preference due to diurnal variation in nonstructural carbohydrates, with increased preference in afternoon cut hay in comparison to morning cut hay associated with increased TNC and in vitro true dry matter disappearance, and decreased neutral-detergent fiber.

Seasonal variation is also documented for nonstructural carbohydrate concentrations in forages. Pollock and Jones (1979) measured fructan metabolism monthly in the cool-season grasses timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.), and perennial ryegrass (*Lolium perenne* L.). They found that, over the course of a full year, fructan accumulation was highest in fall and winter, with maximal values occurring in December, when growth was restricted but photosynthesis continued. Cubitt et al. (2007) documented highest concentrations of nonstructural carbohydrates of Kentucky bluegrass (*Poa pratensis* L.) and tall fescue occurring in the spring and fall months. Kagan et al. (2011) found that both pasture and hay samples followed seasonal trends in the spring, with highest levels in early April and a decrease to lowest levels in May and June. The observed trends indicate that seasonal fluctuations in nonstructural carbohydrate concentrations are correlated with plant growth stages. Young, rapidly growing grasses typically accumulate less nonstructural carbohydrate because of the energy needed to produce new growth. Therefore, as with season, maturity stages will largely affect nonstructural carbohydrate concentrations. They will also determine the point in a season at which a particular forage will reach its maximum nonstructural carbohydrate concentrations. Jensen et al. (2014) reported that two species that were planted at the same time reached their maximum carbohydrate concentrations a month apart; early maturing creeping meadow foxtail reached its peak in the beginning of May while late maturing tall

wheatgrass peaked in the beginning of June. Waite and Boyd (1953) studied fructan accumulation from April to October in tall fescue, orchardgrass and timothy, and found seasonal fructan peaks in May and July/August. They associated these peaks in production with flowering initiation and development, and with carbohydrate formation in the seed (Waite and Boyd, 1953).

Variation from Environment

Environmental conditions such as temperature, precipitation and photosynthetically active radiation (PAR) can affect nonstructural carbohydrate production and utilization. When plant stresses such as extreme temperatures or water deficiencies slow growth rates more than photosynthetic rates, carbohydrate accumulation can occur (Watts and Chatterton, 2004; Longland and Byrd, 2006). Studies with darnel ryegrass (*Lolium temulentum* L.) demonstrated that when photosynthesis continued during times that were too cold for plant growth, carbohydrate accumulation occurred and concentrations were higher at cooler temperatures (Pollock et al., 1983). Labhart et al. (1983) also observed higher WSC concentrations in meadow fescue at 11 to 16 ^{o}C than at 21 to 26 ^{o}C. Brown and Blaser (1965) studied how factors in the environment, including nitrogen availability, temperature and rainfall, affect growth rate and accumulation of reserve carbohydrates in tall fescue and orchardgrass. They found that carbohydrate accumulation occurred under conditions that reduced grass growth, such as low temperature and moisture.

While cooler temperatures (5-10 ^{o}C) have been associated with higher carbohydrate concentrations, warmer temperatures (15-25 ^{o}C) have been associated with low carbohydrate concentrations (Chatterton et al., 1989). Reserve carbohydrates will decrease under conditions where forage growth rates are higher than photosynthetic rates (Longland and Byrd, 2006). In the Brown and Blaser (1965) study, carbohydrates either decreased or remained at low concentrations under temperature and rainfall conditions that promoted grass growth. Waite and Boyd (1953) saw low fructan concentrations during periods of increased growth after rising temperatures due to utilization of carbohydrate reserves. Kagan et al. (2011) also measured decreases in nonstructural carbohydrate concentrations that could have been due to sampling days preceded by one to two weeks of increased

temperatures. Temperature also plays a role in seasonal variation. While peak periods of nonstructural carbohydrate accumulation tend to be in spring and fall months, the summer months typically demonstrate a drop in nonstructural carbohydrate concentrations (Cubitt et al., 2007). This drop occurs because while nonstructural carbohydrates are still being produced throughout the day, temperatures have increased, increasing respiration and therefore utilizing higher amounts of nonstructural carbohydrates for maintenance and growth.

In addition to temperature, PAR has a significant impact on nonstructural carbohydrate concentrations. Low PAR generally results in decreased nonstructural carbohydrate concentrations, and high PAR typically results in nonstructural carbohydrate accumulation. Labhart et al. (1983) discovered that high PAR led to higher WSC in both the stem and leaf tissue of the cool-season grass meadow fescue. Ciavarella et al. (2000) measured lower nonstructural carbohydrate concentrations of forage in shaded areas when compared to non-shaded areas. In a study by Kagan et al. (2011), similar WSC and ESC concentrations were observed in both morning and afternoon samples from one sampling day. This similarity was suggested to be due to low PAR prior to the sampling day, leading to lower than usual afternoon concentrations. Precipitation, or lack thereof, will also affect nonstructural carbohydrate concentrations. Volaire and Lelièvre (1997) observed significant increases of WSC in orchardgrass cultivars during a three month drought, reaching 350-415 g/kg dry matter in the stem base by the end of the drought, and a 40% increase in WSC.

Variation from Genotype

Nonstructural carbohydrate concentrations vary with species and cultivar. Chatterton et al. (1989) depicted significant species differences in TNC, fructan, sucrose, glucose, fructose, and starch among 185 Gramineae species, as well as species by temperature interactions. In this study, tall fescue was among the higher TNC cool-season species, and Kentucky bluegrass was among the lower TNC species (Chatterton et al., 1989). In 2014, Jensen et al. tested 17 species in an irrigated pasture in northern Utah. In this study, perennial ryegrass and timothy contained the highest TNC while meadow

bromegrass contained the lowest TNC (Jensen et al., 2014). Some breeders are even selecting cultivars for low or high nonstructural carbohydrate concentrations. For example, the Welsh Plant Breeding Station in Aberystwyth, Wales, has developed cultivars of perennial ryegrass with high and low WSC (Humphreys, 1989; Wilkins and Humphreys, 2003). Humphreys (1989) documented a range of WSC concentrations in perennial ryegrass cultivars of 13-22%.

Additionally, the amount of fructan present as a percentage of WSC can vary with species and cultivar. Jensen et al. (2014) observed large variations in fructan among species; perennial ryegrass, crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] and Kentucky bluegrass showed the highest percentage of fructan in WSC, at 54%, 47% and 42%, respectively, with the lowest concentrations of 26% and 29% in tall wheatgrass and creeping meadow foxtail, respectively. Shewmaker et al. (2006) documented variation in the proportion of fructan, sucrose, glucose and starch in TNC for eight tall fescue cultivars on four sampling dates from mid-May to mid-September. They found that the proportion of carbohydrate fractions in TNC varied among cultivars but that the rate of accumulation was consistent across all cultivars. They also saw that the percentage of fructan in TNC for tall fescue was highest in July, but that sucrose was the largest contributor to TNC for the rest of the sampling dates, including variance in production of carbohydrate fractions across the season (Shewmaker et al., 2006).

Variation from Management

Management such as defoliation, fertilizer applications and herbicide use can affect nonstructural carbohydrate concentrations in pastures. In general, management that stimulates growth will utilize reserve carbohydrates and decrease nonstructural carbohydrate concentrations in the plant. Lacey et al. (1994) found that monthly defoliation of spotted knapweed (*Centaurea maculosa* Lam.) decreased WSC concentrations by 50%. Another study showed that defoliation decreased the amount of sucrose and fructan in the lower stem and roots of orchardgrass in proportion to their concentration before defoliation; particularly in the roots where initial concentrations of sucrose were high, greater decreases occurred (Sprague and Sullivan, 1950). However, in this study, after the

initial drop in sucrose and fructan concentrations after defoliation, initial concentrations were restored before the next cutting 35 days later (Sprague and Sullivan, 1950). Fertilizer applications can have the same effect by increasing forage growth and utilizing carbohydrates. Studies have documented lower WSC (Jacobs et al., 1989) and fructan in response to increased forage growth after nitrogen applications. Brown and Blaser (1965) found that under low nitrogen, carbohydrates accumulated, whereas under high nitrogen, carbohydrate concentrations were lower. Sprague and Sullivan (1950) reported that, in general, fructan and sucrose concentrations were lower under high nitrogen applications than under low nitrogen applications. High nitrogen stimulates growth more so than low nitrogen, so more reserve carbohydrates would be used. Their study also suggests that utilization of simple sugars occurs only when simple sugar concentrations are high, but not at lower concentrations. Sucrose utilization, in particular, was greater when initial sucrose concentrations were high (Sprague and Sullivan, 1950). However, they also saw that the effects of nitrogen vary with time after cutting and the number of cuts, as well as variation with simple sugars; sometimes simple sugars were higher under high nitrogen applications, while at other times there was no difference (Sprague and Sullivan, 1950). Lechtenburg et al. (1972) observed decreases in fructan after nitrogen fertilization from 7.3 to 0.4% in the spring and from 1.6 to 0.5% in the summer, on a dry matter basis. In the spring, sucrose was decreased by nitrogen fertilization, and the unfertilized forage accumulated fructan (Lechtenburg et al., 1972). Exposure to herbicides or heavy metals can also affect nonstructural carbohydrate concentrations. Engle and Bonham (1980) studied total nonstructural carbohydrates in roots of gambel oak sprouts following herbicide treatments, and saw that several herbicide treatments significantly increased seasonal mean TNC when compared to the controls. In a study by Frossard et al. (1989), fructan was increased in the shoots of ryegrass by 25% with the application of cadmium and nickel, and by 42% and 188% with the application of copper and zinc, respectively.

Summary of Factors Affecting Nonstructural Carbohydrate Accumulation in Forages

In summary, nonstructural carbohydrates in forages vary diurnally and seasonally, as well as with genotype, management and environment. Nonstructural carbohydrate

concentrations are typically higher in the afternoon than in the morning, and peak periods of nonstructural carbohydrate accumulation occur in the spring and fall. Environmental conditions that favor growth, such as high temperatures and rainfall, will utilize reserve carbohydrates for growth and therefore reduce nonstructural carbohydrate concentrations. Environmental conditions such as low temperatures and no rainfall that reduce growth but still allow photosynthetic activity will result in nonstructural carbohydrate accumulation. High PAR typically causes nonstructural carbohydrate accumulation while low PAR typically reduces the amount of nonstructural carbohydrates. Nonstructural carbohydrate concentrations are reliant on an interaction of factors in the environment, so these general patterns may vary. For example, the summer months typically have very high PAR, but nonstructural carbohydrate concentrations tend to be lower in the summer when compared to the fall and spring. These lower concentrations in the summer are due to high nighttime temperatures that increase respiration, utilizing more nonstructural carbohydrates and resulting in lower nonstructural carbohydrate concentrations. There are significant species and cultivar differences as well, and cultivars are being developed for high or low nonstructural carbohydrate concentrations. Management of pastures will also affect nonstructural carbohydrate concentrations. Management tools that stimulate growth, such as fertilizer applications, can result in decreased nonstructural carbohydrate concentrations, while management tools that reduce growth but do not affect photosynthesis can cause nonstructural carbohydrate accumulation.

1.4 Nonstructural Carbohydrates and Pasture-associated Equine Laminitis

Nonstructural carbohydrates are necessary for animal maintenance, growth and development, and can be consumed from concentrate, dried forage feeds and pastures. Most grazing animals spend several hours a day grazing pastures, consuming variable amounts of nonstructural carbohydrates. Some animals may require a high or low nonstructural carbohydrate diet for optimal health and production. Grazing animals such as high performance cattle that have high nutrient demands typically require a diet high in nonstructural carbohydrates (Fisher et al., 1999), however, horses can be sensitive to

excessive amounts of nonstructural carbohydrates when consumed over a short period of time (Longland and Byrd, 2006).

Impacts of Equine Laminitis

Many clinical studies have linked the consumption of excessive amounts of nonstructural carbohydrates, from either pasture or concentrate, with inducing laminitis (Pollitt and Milinovich, 2017). Equine laminitis is a debilitating condition that leads to the detachment of the distal phalanx (also referred to as the coffin bone or the pedal bone) to the laminae of the inner hoof wall, resulting in severe pain and lameness (Pollitt, 2004). In many cases a downward rotation of the distal phalanx occurs (Figure 1.1). Laminitis is a devastating disease and is well known in the horse industry because 20-30% of cases lead to the end of a horse's career or euthanasia (Redden, 2005). It is said that few diseases cause more of an emotional response from horse owners, trainers, veterinarians and farriers due to the extreme pain to the horse and sometimes mortality (Belknap, 2017). The disease of equine laminitis is fraught with controversy (similar to human medical conditions such as cancer) surrounding the complexity and unforgiving nature of the disease, ongoing research on the pathogenesis, and effective treatment options (Belknap, 2017). Laminitis can occur in any horse, regardless of breed and discipline; however, it is mostly seen in mature horses (Redden, 2005). It can cause catastrophic damage and should always be treated as an emergency (Redden, 2005). Fortunately, equine laminitis cases range in severity, and in many cases, especially if they are mild, horses recover with treatment (Redden, 2005).

The most common form of laminitis is pasture-associated laminitis (PAL), or laminitis resulting from "grazing lush pastures" (Kane et al., 2000), and is known as the endocrinopathic form of laminitis (Pollitt and Milinovich, 2017; Walsh and Burns, 2017). Forty-six percent of laminitis cases in the United States have been diagnosed as pasture-associated laminitis by the 1998 National Animal Health Monitoring System (Kane et al., 2000), and there is strong evidence linking PAL with excessive intakes of non-structural carbohydrates, particularly fructan (Pollitt and Milinovich, 2017).

Physiology of Pasture-associated Equine Laminitis

PAL is caused by consuming excessive amount of nonstructural carbohydrates within a short period of time (Pollitt and Milinovich, 2017). Sucrose, glucose, fructose and starch are digested by mammalian enzymes in the stomach and small intestine (Pollitt and Milinovich, 2017). Blood sugar levels are then increased and insulin is released from the pancreas (Pollitt and Milinovich, 2017). For horses that are insulin-resistant, blood-insulin concentrations can exceed the threshold levels and induce laminitis (Pollitt and Milinovich, 2017). Therefore, insulin resistance has been linked specifically to laminitis in horses. In insulin-resistant horses, excessive intake of nonstructural carbohydrates can not only cause laminitis but intensify insulin resistance as well (Hoffman et al., 2003). The simple sugars fructose (Geor et al., 2010) and glucose (Borer et al., 2012) have been identified specifically as intensifying insulin resistance in previously laminitic horses.

Fructans, long-chain fructose polymers, are not digested by mammalian enzymes (Nilsson et al., 1988), and it is believed that most fructans make it to the large intestine (the cecum and the colon, also called the hindgut) without being broken down or digested (Longland and Byrd, 2006). Enzymatic digestion of starch is limited in the small intestine, and concentrations that exceed these limits can be pushed to the large intestine undigested as well. When high concentrations of starch and/or fructan reach the hindgut, billions of microbes (the hindgut microbiome) digest it for the horse (Pollitt and Milinovich, 2017), known as fermentation. This process proliferates amylolytic and saccharolytic bacteria. These bacteria produce lactic acid, thus lowering pH in the hindgut (Longland and Byrd, 2006). Rapid fermentation in the hindgut can disturb the microbiome (Geor, 2010), and combined with reduced pH, can cause a cascade of events including hindgut acidosis, and results in compromised blood flow to the hoof capsule (Longland and Byrd, 2006).

Reduced blood supply, or ischemia, to the foot of the horse can lead to inflammation of the laminae in the hoof capsule and subsequent cell death, since the laminae cannot obtain enough necessary nutrients from the blood supply (Redden, 2005). Ultimately, this deteriorates the laminae (Redden, 2005). The laminae connecting the distal phalanx to the hoof wall acts similar to Velcro strips holding the two pieces of fabric

together. Therefore, severity depends on how much surface area of the laminae was destroyed (Redden, 2005). Current research on the vascular pathophysiology of this mechanism are beginning to investigate endothelial dysfunction as playing a role rather than just lamellar ischemia (Walsh and Burns, 2017).

Laminitis has been induced experimentally by administering high concentrations of starch (Garner et al., 1977) and fructan (Van Eps and Pollitt, 2006). Van Eps and Pollitt (2006) used commercially available fructan extracted from the roots of chicory (*Cichorium intybus* L.) as an oligofructose model to induce laminitis. Oligofructose consists of short-chain, inulin-like fructose polymers intended to mimic fructan in temperate grasses (Longland and Byrd, 2006). Fructans with lower DP, or shorter chain length, are more rapidly fermented in the hindgut, and therefore may pose a higher risk than long chain fructans (Geor, 2010). In the Van Eps and Pollitt (2006) study, the oligofructose model induced laminitis in all horses receiving the treatment 24-36 hours post administration, given at 7.5, 10, and 12.5 g kg^{-1} body weight. They concluded that there is indeed a link between fructan consumed from pasture and pasture-associated laminitis.

It is typically assumed that horses consume dry matter at a rate of around 2% of their body weight per day (Pollitt and Milinovich, 2017). However, this can be an underestimation as horses grazing pasture 12-17 hours per day can consume up to 5% body weight per day (Longland et al., 2016). Longland et al. (2016) observed that horses can consume up to 1% their body weight within the first three hours grazing pasture under certain conditions. This observation was made during the fall when pastures were particularly palatable. This study also observed variable rates of water-soluble carbohydrates (sucrose, glucose, fructose, and fructan), intake throughout the growing season (Longland et al., 2016). For example, water-soluble carbohydrate intake was significantly higher in the fall than during the summer. This observation makes sense since water-soluble carbohydrates in temperate grasses are typically higher in the fall than during the summer. Walsh and Burns (2017) also noted that laminitis cases tend to be higher in the spring and fall, correlating with periods that are historically high for nonstructural carbohydrates. In pastures with high nonstructural carbohydrate concentrations, and high levels of intake (2.5 and 5.2% body weight per day), horses could consume between 3.5

and 7.3 kg fructan per day, respectively (Pollitt and Milinovich, 2017). (These values would also depend on the actual concentration of fructan in the pasture). When compared to the amount of oligofructose used in the previous study, these numbers are similar to and almost double the amount needed to induce laminitis (Pollitt and Milinovich, 2017). However, it's important to remember that in studies inducing laminitis with a bolus, the dose is administered at one time, reaching the cecum 2 hours after administration, peaking at 4 hours and taking 32 hours for full fermentation (Pollitt and Milinovich, 2017). When horses graze pastures, fructan reaches the hindgut throughout the day or grazing period. Frank et al. (2010b) recommended that the total TNC in the diet should be less than 10% on a dry matter basis for horses at risk for laminitis or other metabolic conditions.

1.5 Analytical Procedures

Wet Chemistry Procedures

Nonstructural carbohydrates, excluding starch, can be quantified in terms of water-soluble carbohydrates (WSC) or ethanol-soluble carbohydrates (ESC). As mentioned in section 1.1, ethanol or ethanol-water mixtures primarily extract simple sugars (glucose, fructose and sucrose) whereas water extracts simple sugars and fructan. Some studies have shown that ethanol can extract some short chain fructans in addition to simple sugars (Pavis et al., 2001), therefore the difference between ESC and WSC consists mostly of long chain fructans. Quantifying WSC and ESC requires an extraction followed by a colorimetric assay. Two common colorimetric assays used are the phenol-sulfuric acid assay (DuBois et al., 1956) and the potassium ferricyanide assay (Hulme and Narain, 1931; Brushwood, 2000).

Near Infrared Reflectance Spectroscopy (NIRS)

Wet chemistry procedures alone can be costly and time consuming depending on the number of samples required. In addition to colorimetric assays, near infrared reflectance spectroscopy (NIRS) has also been used to quantify nonstructural carbohydrate

concentrations. NIRS was developed as a time efficient, low-expense tool for forage and feed analysis, among many other uses in research and industry. Advantages of developing NIRS from wet chemistry data, instead of relying on wet chemistry alone, include large sample capacity, minimal preparation of samples, time efficiency, relatively low cost per sample and ease of use.

The History of NIRS

Historically, forage has been marketed and utilized by appearance and weight (Shenk and Westerhaus, 1994). During the past 100 years of research, nutritional analysis has become an important indicator of forage quality and essential in formulating feed rations. During this time, many wet chemistry procedures were developed to predict forage quality including neutral-detergent fiber (NDF), acid-detergent fiber (ADF), and a range of *in vitro* fermentation methods. More recently, NIRS was developed as a more time efficient method.

The near infrared spectrum was first discovered in the 1800s by William F. Hershel, with first reports of near infrared reflectance spectroscopy in the literature in 1939 by Gordy and Martin (Shenk and Westerhaus, 1994). In 1968, NIRS utilization began for analysis of agricultural products by Ben-Gera and Norris, and 10 years later the USDA NIRS Forage Network was initiated for software development and research. NIRS production reached commercial companies in 1983, who marketed the NIRS instrument for forage and feed analysis. Since then NIRS has been used in agriculture including nutritional quality analysis of forage and feed (Marten et al., 1989), food crops (Delwiche, 2004; Dyer, 2004; Hartwig, 2004; Sekiguchi et al., 2004; Slaughter and Abbott, 2004;), and human food industries (Dambergs et al., 2004; Downey and Hildrum, 2004; Garrido-Varo et al., 2004; Giangiacomo and Cattaneo, 2004; Sandra, 2004; Scotter and Miller, 2004). In addition to agriculture, NIRS is used in a variety of industries including pharmaceuticals (Ciurczak, 2002) and human medicine (Strangman et al., 2002; Murkin and Argano, 2009).

The NIRS Technique

The infrared region of the electromagnetic spectrum is broken up into near, middle, and far infrared sections. The near infrared region gets its name from being the "nearest" infrared region to the visible region. The NIRS reads the absorbance of each sample in the visible region (400-1100 nm) and near infrared region (1100-2500 nm) which is then used to develop a unique spectrum (Ruiz, 2001). The NIRS develops a spectrum by focusing a laser of near infrared light through a sample, where, depending on the chemical bonds present, some light is absorbed and some is reflected back to the detector. Compounds absorb NIR radiation at specific wavelengths; compounds high in N-H (such as proteins and amino acids), O-H (moisture, carbohydrates, and fat), and C-H bonds (organic compounds) are absorbed at wavelengths which occur in the near infrared region (Figure 2). These molecular bonds are used to develop the unique spectrum for each sample, which serves as a composite of all absorbencies from all bonds in the sample (Ruiz, 2001), essentially creating a chemical fingerprint of the sample. The NIRS technique of calibrated equations uses a sample's spectrum combined with wet chemistry values of a specific compound to predict concentrations of that compound in unknown samples. Calibration equations are developed by combining NIRS spectra with coordinating wet chemistry data of a population representative to the samples that will be analyzed. These two sets of data create a mathematical relationship that can then be used to predict compounds in unknown samples. Accuracy in calibration development with known samples is crucial for accuracy of prediction of unknown samples.

The Global H value (GH) and the Neighborhood H value (NH) are used in calibrating equations to estimate variance. The GH is an estimation of concentrations that occur outside of the population of concentrations used in calibration development. Typically, concentrations with a GH greater than three are considered outliers, whereas concentrations with a GH less than three are similar to the population. The NH identifies the distance each spectrum is from every other spectra (Ruiz, 2001). A NH less than six indicates that the sample's spectrum is very close to the spectral population, therefore resulting in a reliable, robust calibration equation (Ruiz, 2001).

In summary, the NIRS has evolved as a valuable tool to estimate forage quality or other parameters. Advantages include being able to handle a large sample size, little preparation requirement of samples (depending on the material), efficiency and ease of use. It has been especially effective in studies requiring large sample populations, which nonstructural carbohydrate studies often do. Wilkinson et al. (2014) used NIRS equations calibrated with wet chemistry to quantify WSC for 8814 samples over the course of seven years. They saw a large range of concentrations and recommended taking weekly samples for accuracy in management and developing feed rations (Wilkinson et al., 2014). Shetty et al. (2012) used NIRS in combination with chemometrics to quantify TNC in grasses over a period of five years. They determined that to reduce year-to-year variation, new samples should be added to the equation each year. The equation should be recalibrated including the new samples, and then used to predict unknown samples (Shetty et al., 2012).

1.6 Objectives and Hypothesis

Understanding and controlling nonstructural carbohydrate intake, particularly fructan, in grazing horses is key to not only managing an appropriate nutritional diet, but in managing and preventing pasture-associated laminitis in at-risk horses. Nonstructural carbohydrates in cool-season grasses of pastures are affected by many factors and have the potential to change very quickly. It is important to understand nonstructural carbohydrate patterns of accumulation in cool-season grasses as well as to be able to quantify them. Having a better understanding of how nonstructural carbohydrates vary in pastures will help manage animals that require a high or low nonstructural carbohydrate diet. The objectives of this study were 1) to evaluate the effects of genotype, management, and environment on WSC and ESC in cool-season grass pastures in central Kentucky, and 2) to develop robust near-infrared reflectance spectroscopy (NIRS) calibrated equations for faster and easier analysis of WSC and ESC in cool-season grasses. Faster analysis will aid in monitoring and understanding nonstructural carbohydrate patterns in pastures, ultimately leading to better pasture and animal management.

Figure 1.1 Changes that occur to the equine foot as a result of laminitis, with the laminitic foot on the right and a normal foot on the left. (Pavord and Pavord, 2005)

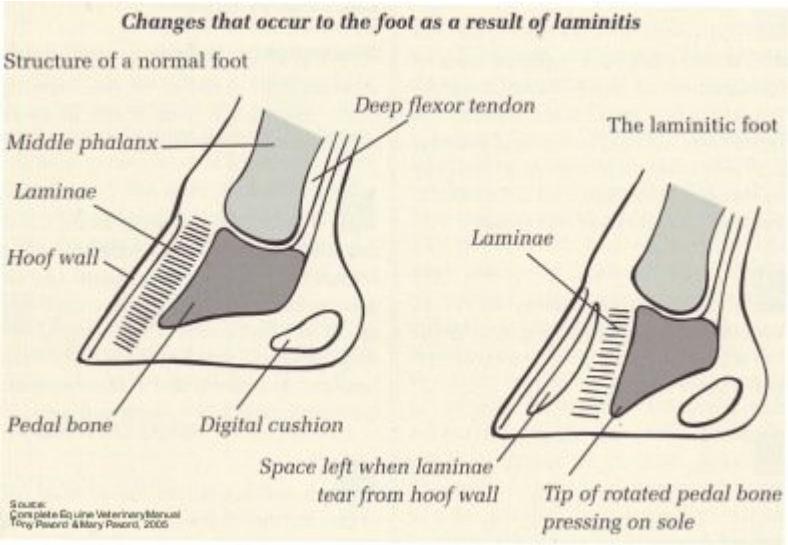
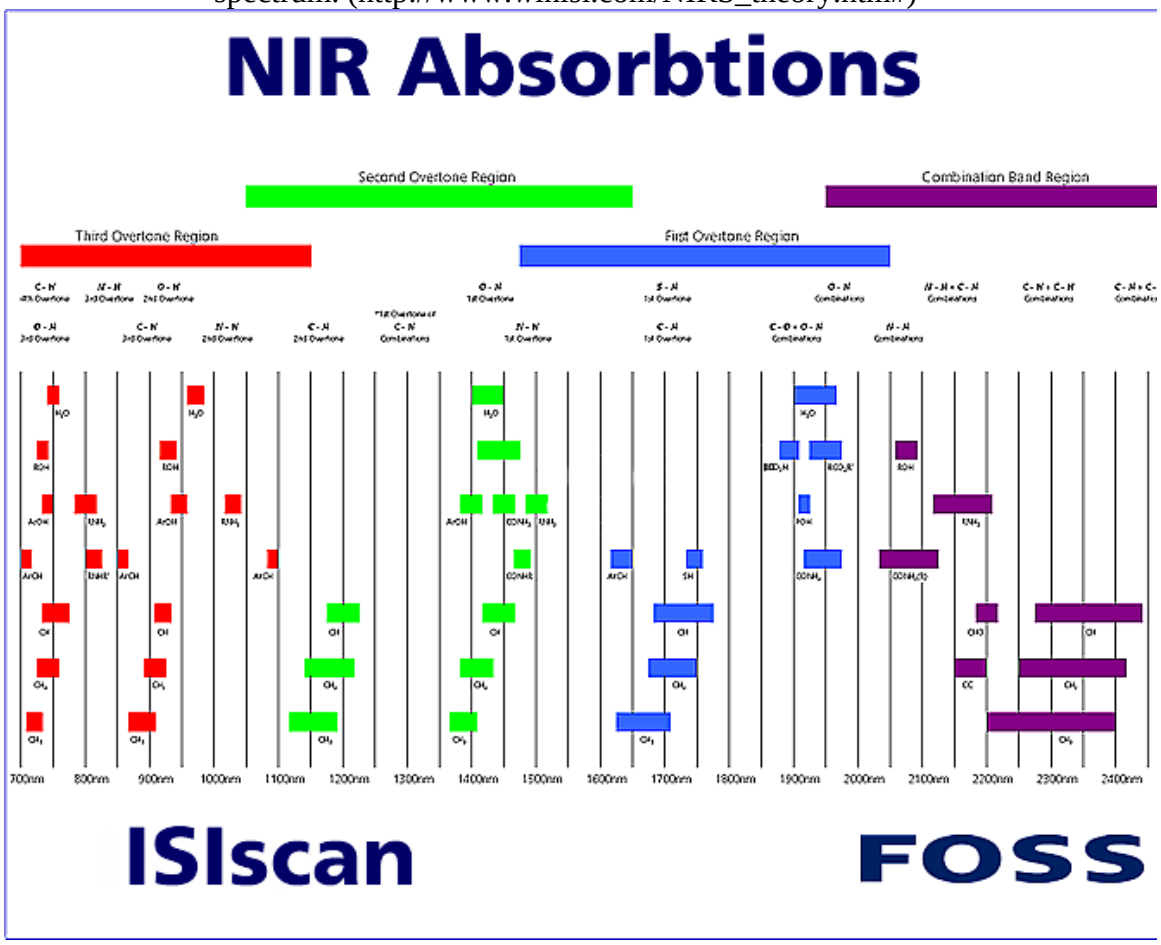


Figure 1.2 Chemical bond absorptions at various wavelengths through the near infrared spectrum. (http://www.winisi.com/NIRS_theory.htm#)



Chapter Two

Predicting Water-soluble Carbohydrates and Ethanol-soluble carbohydrates in Cool-season Grasses with Near Infrared Reflectance Spectroscopy

2.1 Abstract

Understanding how nonstructural carbohydrate concentrations fluctuate in pastures and being able to quantify them is an essential component to successfully managing grazing animals. Relying entirely on laboratory chemistry to quantify nonstructural carbohydrates can be time consuming and costly. The objective of this study was to develop near infrared reflectance spectroscopy (NIRS) calibration equations for analysis of water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC) in cool-season grasses. Ten cool-season grass cultivars consisting of Kentucky bluegrass, tall fescue, orchardgrass and perennial ryegrass were sampled in the morning and afternoon every two to four weeks during the growing season from the University of Kentucky Research Farm in Lexington, KY. Samples were immediately flash frozen after sampling, freeze dried, ground, and scanned into FOSS 6500 NIRS with ISIscan software. WSC and ESC for a subset of samples were determined using colorimetric phenol-sulfuric acid assays and served as the basis for NIRS calibration. NIRS equations for both WSC and ESC accurately predicted wet chemistry values for a wide range of values, ranging from 2% to 20%. Both equations also had high 1-VR and R^2 values of over .90 and .93, respectively, indicating strong predictability. This method will aid in timely monitoring of nonstructural carbohydrate concentrations in pastures in the future, and will allow for the more efficient management of grazing animals.

2.2 Introduction

Many clinical studies have linked the consumption of excessive amounts of nonstructural carbohydrates with inducing equine laminitis (Pollitt and Milinovich, 2017). Equine laminitis is a debilitating condition that leads to the detachment of the distal phalanx to the

laminae of the inner hoof wall, resulting in severe pain and lameness (Pollitt, 2004). Laminitis is well known in the horse industry where approximately 20-30% of laminitis cases see devastating results, ending the horse's career or requiring euthanasia (Redden, 2005). Forty-six percent of laminitis cases in the United States have been diagnosed as pasture-associated laminitis (PAL) by the 1998 National Animal Health Monitoring System (Kane et al., 2000), making it the most common form of laminitis (Pollitt and Milinovich, 2017), and there is strong evidence linking PAL with excessive intakes of non-structural carbohydrates, particularly fructan (Pollitt and Milinovich, 2017). Being able to quantify and monitor nonstructural carbohydrate concentrations in pastures in a timely manner is an important component to combatting this disease in horses. Using only laboratory chemistry to quantify nonstructural carbohydrates of forage pastures can be time consuming and costly.

Near Infrared Reflectance Spectroscopy

Near Infrared Reflectance Spectroscopy (NIRS) has been developed as a valuable tool to estimate forage quality or other parameters. Advantages include being able to handle a large sample size, little preparation requirement of samples (depending on the material), efficiency and ease of use. It has been especially effective in studies requiring large sample populations, which nonstructural carbohydrate studies often do. Wilkinson et al. (2014) used NIRS equations calibrated with wet chemistry to quantify WSC for 8814 samples over the course of seven years. They saw a large range of concentrations and recommending taking weekly samples for accuracy in management and developing feed rations (Wilkinson et al., 2014). Shetty et al. (2012) used NIRS in combination with chemometrics to quantify TNC in grasses over a period of five years. They determined that to reduce year-to-year variation, new samples should be added to the equation each year. The equation should be recalibrated including the new samples, and then used to predict unknown samples (Shetty et al., 2012).

The objective of this study was to develop near infrared reflectance spectroscopy (NIRS) calibration equations for analysis of water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC) in cool-season grasses so that they can be used in

future research and eventually be available to animal managers. ESC includes simple sugars (glucose, sucrose, and fructose), whereas WSC includes simple sugars and long-chain fructans. Some studies have shown that ethanol can extract some short chain fructans in addition to simple sugars (Pavis et al., 2001), therefore the difference between ESC and WSC consists mostly of long chain fructans.

2.3 Materials and Methods

Forage Sampling

Calibration development used two sets of data that occurred over two years of sampling. The first year of data, referred to as sample set 1, used cool-season grass samples collected in 2014 to begin building the database for WSC and ESC equations. This study sampled eight cultivars planted in a randomized complete block design representing four species: Kentucky bluegrass (*Poa pratensis* L.) 'Barderby' and 'Ginger', orchardgrass (*Dactylis glomerata* L.) 'Persist' and 'Profit', tall fescue (*Festuca arundinacea* Schreb.) 'Cajun' and 'Bronson', and perennial ryegrass (*Lolium perenne* L.) 'Calibra' and 'Linn' from the Forage Variety Trial (Olson et al., 2014a,b,c,d) plots located on the University of Kentucky Research Farm (Lexington, KY). Samples were collected every 7-14 days from April 18, 2014 to May 30, 2014, and ranged in maturity from vegetative to seedhead stage. A fall harvest was also collected on October 17, 2014, with all samples in the vegetative stage of maturity.

Samples were collected by clipping approximately 100 g of forage at 5 cm forage height from several random locations throughout each plot to mimic grazing. Forage was then diced with garden shears to 2.5 to 5 cm length before being placed in a 20 cm diameter aluminum collecting pan. This procedure was repeated at five to ten random locations throughout each plot, until approximately 100 g of forage had been collected. Samples were immediately flash frozen by placing the pan in Styrofoam coolers that held enough liquid nitrogen to cover the bottom and sides of the collecting pan. Samples were transported in coolers with dry ice to -20 ^{o}C freezers where they were stored until being freeze dried. Samples were freeze dried for seven to ten days from -29 ^{o}C to 23 ^{o}C. Three

freeze dryers were used to accommodate the large number of samples: Botanique (Phoenix, AZ) model 18DX48SA, VirTis SP Industries (Gardiner, NY) model 36X66GPFD, and Botanique (Phoenix, AZ) model 24DX54. Once samples were freeze dried, they were ground using a 1mm mesh in an Udy Cyclone Sample Mill (UDY Corporation, Fort Collins, CO). They were then scanned into a Foss 6500 Near Infrared Reflectance Spectroscopy (NIRS) (Foss, Inc., Hillderod, Denmark) with ISI software (Infrasoft International, L.L.C., State College, PA).

Sample set 2 consisted of samples collected in 2015 from seeded research plots. These plots were seeded 8 September 2014 and consisted of 10 cultivars representing four species: Kentucky bluegrass ('Barderby' and 'Ginger'), orchardgrass ('Persist,' 'Profit' and 'Quickdraw'), tall fescue ('Cajun II' and 'Bronson'), and perennial ryegrass ('Calibra,' 'Linn' and 'Aberzest'). The experimental design was a randomized complete block, split-block, design. Nitrogen (N) treatments (0 and 157 kg N ha^{-1}) were applied as split-blocks to evaluate carbohydrate variation from soil fertility. The nitrogen treatments were applied as split applications: 56.07 kg N ha^{-1} (16 March 2015), 39.23 kg N ha^{-1} (13 May 2015), and 56.07 kg N ha^{-1} (19 August 2015) with a Gandy 3 ft (Owatonna, MN) drop-type fertilizer spreader.

These plots were sampled twice daily (8:00-9:00 am and 3:00-4:00 pm) every two or four weeks from April 15, 2015 to November 3, 2015. They were mowed to a forage height of 10 to 12.5 cm using a zero turn mower (Kubota ZG227, Torrance, CA) every two weeks, returning forage clippings to pasture to represent a typically managed horse pasture, in which forage is maintained in a vegetative stage at a height of 10 to 25 cm. Other sampling and laboratory methods of the 2015 study were identical to the 2014 study, except that in 2015, to help measure cutting height, polyvinyl chloride (PVC) pipes 5 cm in diameter were used. The PVC pipes were laid down next to the forage being sampled, garden shears were placed on top, and a handful of grass was sampled. Samples from both 2014 and 2015 studies were used in final calibration development to increase population variation and robustness of the equations.

Near Infrared Reflectance Spectroscopy: Selecting Samples for Wet Chemistry

Due to the large sample population of the two studies (n=1873), a smaller population size was chosen for wet chemistry to be used in calibration development. From the 2014 study, samples were chosen at random. Spring 2014 had 168 samples total; 69 of these were selected for WSC and 101 were selected for ESC. From the fall 2014 harvest, 16 of the total 48 samples were chosen for both WSC and ESC. For the 2015 study, samples were selected for wet chemistry using the FOSS 6500 NIRS ISI software so that a sample size was representative of population variance. An H value of 1 was used as selection criteria. The NIRS selected around 13% of the total 2015 population for wet chemistry, averaging 20 samples per harvest.

Thirteen total harvests from 2014 and 2015 were used to develop WSC and ESC equations. Around 16% (n=305) and 18% (n=337) of total samples (n=1874) were used for wet chemistry of WSC and ESC, respectively, and used for calibration development (Table 2.1). In 2015, not all samples from each harvest were available. Due to incomplete freeze drying and subsequent molding of samples, all of harvest 2 and some samples from harvest 3 and 4 were contaminated and therefore not available for selection. In addition, the population size was slightly smaller for the harvest taken on 15 April 2015 because the Kentucky bluegrass samples were not harvested due to limited growth.

Laboratory Procedures

Once samples were selected for wet chemistry, colorimetric, phenol-sulfuric assays were performed to quantify WSC and ESC (DuBois et al., 1956). Samples were weighed to 0.10 g and mixed in 20 x 150 mm test tubes with 20 mL Millipore H_2O for WSC assays and 20 mL 80% ethanol for ESC assays. Tubes were shaken on the FinePCR (Gyeonggi-do, Korea) CR300t rocking shaker at 48 to 53 rpm. WSC extracts were shaken for three hours and ESC extracts were shaken for three to four hours. Tubes were rotated 180^o on the shaker halfway through to reduce pooling of material on the test tube walls. Extracts were filtered with 110 mm filter paper (#4 grade, Whatman, Buckinghamshire, United Kingdom), brought to a final volume of 25 mL with water or 80% EtOH, and mixed

thoroughly. Filtration was done either on the same day as the extraction or after one to two days of storage at 4 ^{o}C. For WSC assays, 10-fold dilutions of filtrate were prepared in H_2O (1 mL extract added to 9 mL H_2O for both WSC and ESC). The final ethanol concentration of the ESC extracts was 8%. Samples were stored at -20 ^{o}C long term or up to two days at 4 ^{o}C prior to assay.

In preparation for colorimetric assays, sampled stored at -20 ^{o}C were thawed overnight at 4 ^{o}C, and then set out at room temperature to finish thawing. WSC samples were sonicated for 5 minutes at 40-45 ^{o}C in the sonicating water bath (model 5510, Branson Ultrasounds Corporation, Danbury, CT). Heating and sonication was used to resuspend long chain fructans that might precipitate when frozen (Chatterton and Harrison, 1997). After sonication, samples were vortexed for 5 seconds at 80-100% power using the Vortex Genie II vortexer. Sonication and vortexing were repeated a second time. Samples were not sonicated or vortexed if assayed without freezing between the extraction and assay procedures because longer chain fructans would not precipitate without freezing and therefore not need to be resuspended (Chatterton and Harrison, 1997).

Colorimetric, phenol-sulfuric acid assays (DuBois et al., 1956), were performed to determine concentrations of WSC and ESC in samples. All samples (average of 20) from a given harvest were assayed for WSC or ESC on the same day. Assays were performed in triplicate. Nonstructural carbohydrates were quantified with a sucrose standard curve, also assayed in triplicate. A stock solution of 2.04 mg/mL sucrose in water was used to prepare the sucrose standard curve for WSC assays. For ESC assays, a stock of 1.08 mg/mL sucrose in 20% EtOH was used, and dilutions were made such that all concentrations were in 8% EtOH (the EtOH concentration of the extracts). Both water and ethanol stocks were serially diluted to 10, 20, 40, and 60 µg/mL. For ESC extracts, 8% EtOH was used as a blank, and for WSC extracts, H_2O was used. The phenol-sulfuric acid assays began with triplicates of 0.5 mL of each sucrose standard in 16 x 100 mm glass tubes. One half mL of unknown sucrose concentrations in samples was also dispensed in triplicates.

In the fume hood, 0.5 mL of 5% (w/w) phenol reagent (Appendix A) was dispensed into each tube, and tubes were then vortexed for three to five seconds. Concentrated sulfuric acid (2.5 mL) was then added. Dispensette III solution dispensers (Brandtech

Scientific, Essex, CT) were used to dispense the phenol and sulfuric acid. Since the addition of sulfuric acid creates an exothermic reaction, tubes were left to cool for ten minutes, then vortexed for three to five seconds.

Samples were then placed into an Edvotek (Washington, DC) water bath, set and verified between 25-30 ^{o}C for 15 minutes. Tubes were immersed with the water line above the level of the assay mixture in the tubes. When finished in the water bath, tubes were dried off and transferred to a dry test tube rack.

The assay mixtures were poured into 4-mL disposable polystyrene cuvettes (Brandtech) until the cuvette was about two thirds full. The absorbance was measured at 490 nm with a spectrophotometer (model DU-800, Beckman Coulter, Fullerton, CA). A second spectrophotometer, BioChrom Libra S4 Spectrophotometer (Cambridge, England), was utilized to accommodate the large number of samples and streamline the process. For this spectrophotometer, samples could be placed directly into the absorbance reader without being transferred into cuvettes, reducing the time needed to transfer them. Reusable instead of disposable glass tubes had to be used in order to minimize variation in readings, possibly due to a more uniform wall thickness in reusable tubes. The sucrose standard curves created a linear regression between sucrose concentration (μg/mL) and absorbance at 490 nm, which was used to calculate unknown sucrose concentrations based on absorbance. Because dry matters were not determined for the freeze-dried samples, calculations were based on the uncorrected sample masses.

Near Infrared Reflectance Spectroscopy: Equation Calibration

Equations were developed using the near-infrared wavelength region (1100-2500 nm) of the electromagnetic spectrum. Samples were originally scanned under both the visible (400-1100 nm) and near-infrared region (1100-2500 nm), however, only the near-infrared region was used due to high variability in the visible region. With the FOSS 6500 NIRS, the visible spectra tends to have more noise (variability) and be less repeatable than the near-infrared spectra (C. Drapcho, FOSS Application Specialist, North America, personal communication). After taking out the visible spectra, the WSC and ESC equations

had reduced errors and increased R^2 (0.93 and 0.97, respectively), indicating that the visible spectra were not adding any positive information to the equation. Also, the commonly used FOSS 5000 NIRS only uses the near-infrared region. Therefore, using only the near-infrared region will make the equations more easily transferrable between machines in the future.

Equations were developed using modified partial least squares regressions, with internal cross-validation, using the groups method with two outlier elimination passes (Shenk and Westerhaus, 1991). A math treatment of 2,5,4,1 was selected to optimize regression statistics with the critical T and GH outlier values of 2.4 and 10, respectively. The scatter used was SNV and detrend with 172 wavelengths, and no X outlier limit was used. Regressions were developed and selected based on high R^2 and 1-VR (The 1-VR value for NIRS is the equivalent of an R^2 for a prediction regression) and low standard errors of calibration (SEC) and cross-validation.

2.4 Results

The WSC concentrations of the calibration samples (n=293), representing 13 total harvests from 2014 and 2015, ranged from 2.43% to 20.13% (Table 2.2). The 1-VR of this equation was 0.90, indicating good predictability of future samples. The ESC calibrations samples (n=323) represents a wide range of estimable ESC concentrations, ranging from 1.70% to 19.90% (on a dry mater basis). The 1-VR for this equation, 0.96, was slightly higher than that of the WSC equation, also indicating good predictability of unknown samples under a wide population range.

To demonstrate the effectiveness of the WSC and ESC equations, the calibration population was predicted by the equations, and plotted as a function of the actual wet chemistry values. These relationships are shown in Figure 2.1 for WSC and Figure 2.2 for ESC. A perfect relationship between actual and predicted concentrations would be indicated by a slope of 1. The regression equation and R^2 values indicate accuracy of the equation in predicting unknown samples. Both equations have slopes above 93% accuracy,

with WSC having a slope of 0.93 and ESC having a slope of .97; both regressions also have a R^2 above .92, with WSC having a R^2 of .92 and ESC having an R^2 of 0.97.

2.5 Discussion

Based on the equation statistics and regressions of predicted values as functions of the actual values, the WSC and ESC NIRS equations showed accurate predictions of wet chemistry values for a wide range of values of 2% to 20% (based on the mass of the freeze-dried tissue). Typically, WSC and ESC for forages fall within this range (Jensen et al., 2014). Wilkinson et al. (2014) built NIRS equations for WSC over the course of seven years, collecting 8814 total samples, and saw similar ranges to the present study. This study had a minimum and maximum of 3% and 24% (on a dry matter basis), respectfully, and an overall mean of 9% (Wilkinson et al., 2014). This study sampled pre-grazed pasture grass from both grassland pastures and dairy farms in the United Kingdom. The authors concluded that for accuracy in management and developing feed rations, it is best to take weekly samples for NIRS calibration development (Wilkinson et al., 2014).

Other studies have also concluded that the best strategy is to build NIRS equations over multiple years, adding to the equation frequently (Shetty et al., 2012). Shetty et al. (2012) used NIRS in combination with chemometrics to quantify TNC in grasses over a period of five years. To test accuracy of the equation, they correlated predicted TNC with measured TNC as demonstrated in the present study. The present study's WSC and ESC equations had r^2 values of 0.92 and 0.97, respectively. Shetty et al. (2012) also observed a high linear correlation with an r^2 of 0.96. They determined that, based on this amount of acceptable low error, the use of NIRS to predict TNC over the course of multiple years is possible. However, to reduce year-to-year variation, new samples should be added to the equation each year. The equation should be recalibrated including the new samples, and then used to predict unknown samples (Shetty et al., 2012). Therefore, in order to maintain accuracy over multiple years in the present study, new samples should be added to validate the equation each year. The equations are currently accurate with acceptable low error in predicting WSC and ESC, and building the equations in future years will reduce year-to-year variation and maintain reliability in application.

While there are commercially available services for predicting WSC and ESC with NIRS methods such as Dairy One (Hall, 2014), the equations from this study are unique in that they are fine-tuned to predict WSC and ESC in cool-season grasses of central Kentucky. They are also unique in that these equations were built on grasses kept in mostly a vegetative state, which is the common management practice for horse pastures in this region. The equations in this study also use consistent sampling and laboratory methods to minimize error. Limitations of the equations in this study include location, fertility and environment. For example, samples used in this study were collected from high fertility pastures on good soils in central Kentucky, growing under the unique environmental conditions of this region.

2.6 Conclusions

The WSC and ESC equations developed from this study provide accurate nonstructural carbohydrate predictions for similarly managed cool-season grass pastures for both research and industry purposes. They will streamline nonstructural carbohydrate analysis and make handling larger sample populations more feasible by minimizing the amount of required wet chemistry analysis, as well as reducing the amount of time it takes to quantify results. WSC and ESC concentrations in pastures have the potential to change very quickly, therefore, faster sample processing time has the potential to improve animal management decisions. Building the equations with additional research samples and using external validation samples from farms would further increase the accuracy and strengthen the reliability of these equations, further improving management decisions for grazing animals.

Table 2.1 Samples selected for water-soluble carbohydrate (WSC) and ethanol-soluble carbohydrate (ESC) equation calibration development by near-infrared reflectance spectroscopy from 2014 and 2015 studies.

Harvest #	Harvest Date	Samples not included	Total n	Selected n for WSC	Selected n for ESC	Selection Method
Spring Harvest 2014	4/18/2014, 4/21/2014, 4/23/2014, 5/6/2014, 5/7/2014, 5/21/2014, 5/30/2014		168	69	101	Random
Fall Harvest 2014	10/17/2014		48	16	16	Random
Harvest 1 2015	4/15/2015	KY Bluegrass	126	21	21	NIRS
Harvest 2 2015	4/29/2015	all	0	0	0	NIRS
Harvest 3 2015	5/13/2015	1.75 out of 4 Reps of AM Harvest	114	20	20	NIRS
Harvest 4 2015	5/27/2015	4 contaminated samples	154	16	16	NIRS
Harvest 5 2015	6/10/2015		158	16	16	NIRS
Harvest 6 2015	6/24/2015		158	24	24	NIRS
Harvest 7 2015	7/8/2015		158	18	18	NIRS
Harvest 8 2015	7/22/2015		158	22	22	NIRS
Harvest 9 2015	8/19/2015		158	21	21	NIRS
Harvest 10 2015	9/15/2015		158	22	22	NIRS
Harvest 11 2015	10/13/2015		158	18	18	NIRS
Harvest 12 2015	11/3/2015		158	22	22	NIRS
ALL HARVESTS			**1874**	**305**	**337**	

Table 2.2 Equation statistics for quantification of water-soluble carbohydrates (WSC, %) and ethanol-soluble carbohydrates (ESC, %) from ten cool-season grass cultivars by near-infrared reflectance spectroscopy (NIRS).

Equation	n	Mean	Range	SEC	RSQ	SECV	1-VR
WSC	293	8.14	2.43-20.13	0.91	0.93	1.07	0.90
ESC	323	7.64	1.70-19.90	0.61	0.97	0.75	0.96

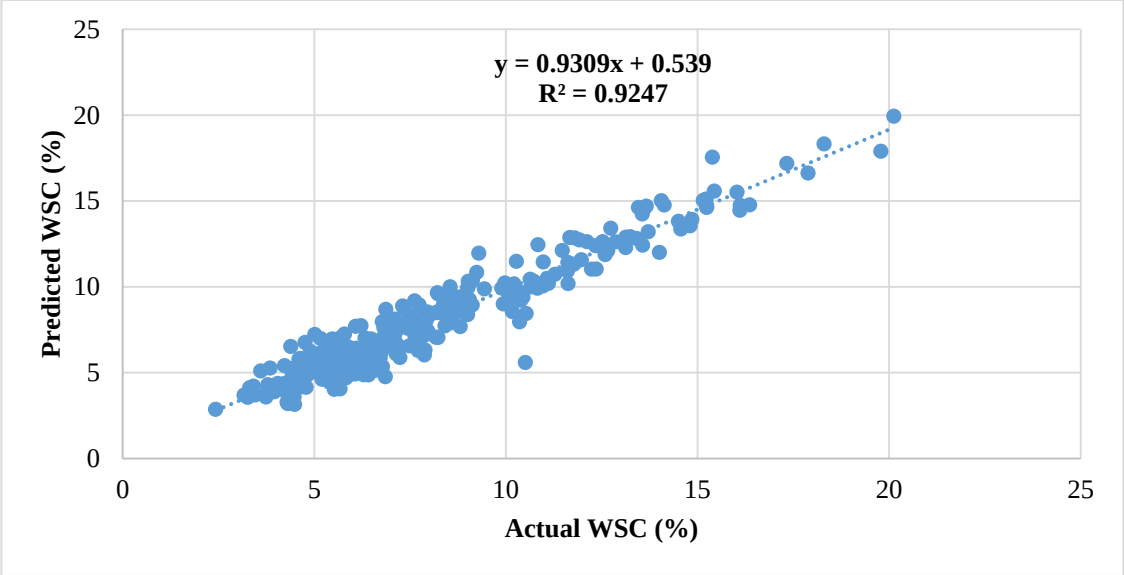


Figure 2.1 Near infrared reflectance spectroscopy equation-predicted water-soluble carbohydrate concentrations (WSC) as a function of laboratory values for ten cool-season grass cultivars, harvested 2014-2015.

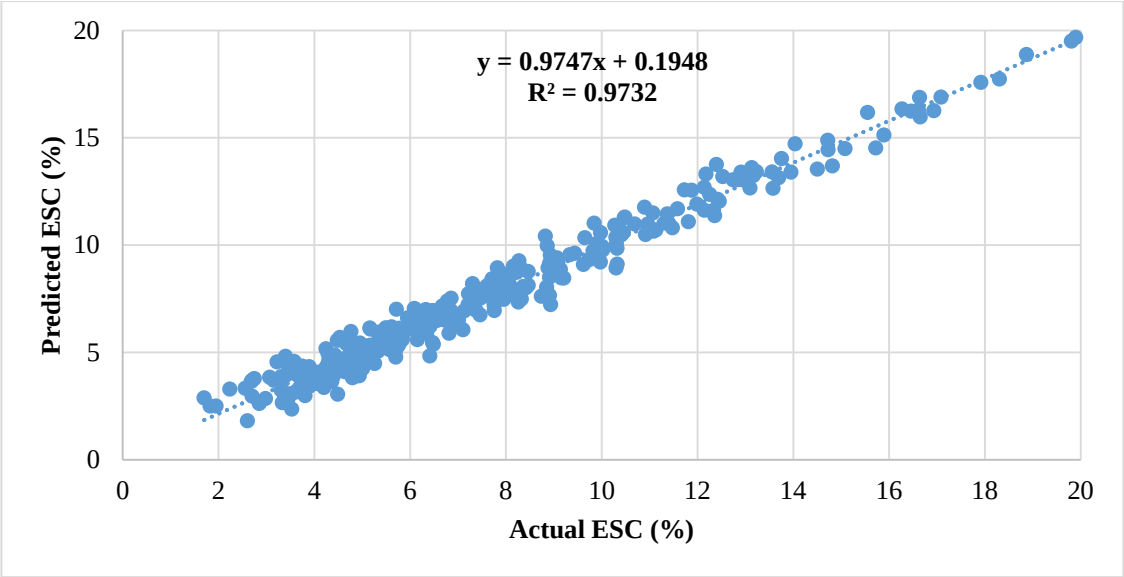


Figure 2.2 Near infrared reflectance spectroscopy equation-predicted ethanol-soluble carbohydrate concentrations (ESC) as a function of laboratory values of ten cool-season grass cultivars, harvested 2014-2015.

Chapter Three

Evaluating the Effects of Species, Cultivar, Harvest Date, Time of Day, and Fertility on Water-soluble Carbohydrates and Ethanol-soluble Carbohydrates of Ten Cool-season Grass Cultivars

3.1 Abstract

Understanding how nonstructural carbohydrates fluctuate in pastures is an essential component to successfully manage grazing animals that may require high or low nonstructural carbohydrate diets. The objective of this study was to evaluate the effects of diurnal and seasonal variation, genotype, and fertility on water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC) in cool-season grass pastures of central Kentucky. Ten cool-season grass cultivars consisting of Kentucky bluegrass, tall fescue, orchardgrass and perennial ryegrass were sampled in the morning and afternoon every two to four weeks during the growing season from the University of Kentucky Research Farm in Lexington, KY. Samples were immediately flash frozen after sampling, freeze dried, ground, and scanned into FOSS 6500 NIRS with ISIscan software. WSC and ESC were determined using colorimetric phenol-sulfuric acid assays on a subset of samples and served as the basis for NIRS calibration. WSC and ESC concentrations were typically highest in the afternoon and lowest in the morning. Perennial ryegrass accumulated the highest WSC concentrations, followed by tall fescue, KY bluegrass, and orchardgrass. Fertility effects were inconsistent among harvest dates and cultivars. WSC and ESC concentrations were generally higher across species under the cooler day and night temperatures during the spring and fall in comparison to the summer. In conclusion, WSC and ESC in cool-season grasses are dependent on an interaction of factors including genotype, management and environment.

3.2 Introduction

Grazing animals such as high performance cattle that have high nutrient demands typically require a diet high in nonstructural carbohydrates (Fisher et al., 1999), however, horses can be sensitive to excessive amounts of nonstructural carbohydrates (Longland and

Byrd, 2006). Frank et al. (2010b) recommends that horses at risk be fed a diet less than 10% TNC on a dry matter basis, as many clinical studies have linked the consumption of excessive amounts of nonstructural carbohydrates with inducing equine laminitis (Pollitt and Milinovich, 2017).

Equine laminitis is a debilitating condition that leads to the detachment of the distal phalanx to the laminae of the inner hoof wall, resulting in severe pain and lameness (Pollitt, 2004). Approximately 20-30% of laminitis cases see devastating results, ending the horse's career or requiring euthanasia (Redden, 2005). Forty-six percent of laminitis cases in the United States have been diagnosed as pasture-associated laminitis (PAL), or laminitis resulting from "grazing lush pastures" (Kane et al., 2000), making it the most common form of laminitis (Pollitt and Milinovich, 2017; Walsh and Burns, 2017).

PAL is caused by consuming excessive amount of nonstructural carbohydrates in a short period of time (Pollitt and Milinovich, 2017). Sucrose, glucose, fructose and starch are digested by mammalian enzymes in the stomach and small intestine (Pollitt and Milinovich, 2017). Blood sugar levels are then increased and insulin is released from the pancreas (Pollitt and Milinovich, 2017). For horses that are insulin-resistant, blood-insulin concentrations can exceed the threshold levels and induce laminitis (Pollitt and Milinovich, 2017). Therefore, insulin resistance has been linked specifically to laminitis in horses. In insulin-resistant horses, excessive intake of nonstructural carbohydrates can not only cause laminitis but intensify insulin resistance as well (Hoffman et al., 2003). The simple sugars fructose (Geor et al., 2010) and glucose (Borer et al., 2012) have been identified specifically as intensifying insuln resistance in previously laminitic horses.

Laminitis has been induced experimentally by administering high concentrations of starch (Garner et al., 1977) and fructan (Van Eps and Pollitt, 2006). Fructans, long-chain fructose polymers, are not digested by mammalian enzymes (Nilsson et al., 1988), and it is believed that most fructans make it to the large intestine without being broken down or digested (Longland and Byrd, 2006). Enzymatic digestion of starch is limited in the small intestine, and concentrations that exceed these limits can be pushed to the large intestine undigested as well. When high concentrations of starch and/or fructan reach the hindgut, billions of microbes digest it for the horse (Pollitt and Milinovich, 2017), known as

fermentation. This process proliferates amylolytic and saccharolytic bacteria, which produce lactic acid, thus lowering the pH in the hindgut (Longland and Byrd, 2006). Rapid fermentation in the hindgut can disturb the microbiome (Geor, 2010), and combined with reduced pH, can cause a cascade of events including hindgut acidosis, and results in compromised blood flow to the hoof capsule (Longland and Byrd, 2006). Reduced blood supply, or ischemia, to the foot of the horse can lead to inflammation of the laminae in the hoof capsule and subsequent cell death, since the laminae cannot obtain enough necessary nutrients from the blood supply, ultimately deteriorating the laminae (Redden, 2005).

Objective of Study

Understanding and controlling nonstructural carbohydrate intake in grazing horses is key to not only managing an appropriate nutritional diet, but also in managing and preventing pasture-associated laminitis in at-risk horses. Nonstructural carbohydrate concentrations in cool-season grasses of pastures are affected by many factors and have the potential to change very quickly. It is important to understand the diurnal and seasonal patterns of nonstructural carbohydrate concentrations in cool-season grass pastures as well as how they are affected by weather conditions and management factors. This knowledge will help animal managers make decisions when trying to provide a high or low nonstructural carbohydrate diet. The objective of this study was to evaluate the effects of genotype, management, and environment on WSC and ESC in cool-season grass pastures in central Kentucky, specifically the effects of species, cultivar, nitrogen fertility, harvest date and time of day.

3.3 Materials and Methods

Forage Establishment and Maintenance

This research was conducted in northern Fayette County, Kentucky on University of Kentucky's North Farm, which serves as an important part of the Kentucky Agricultural Experiment Station. The experimental plot area had a Bluegrass-Maury silt-loam soil type

and was chosen because there was a weather station located at the site. Prior to seeding, the area was sprayed with 2.24 kg glyphosate/ha on 25 July 2014. It was then mowed to 7.5 cm on 30 July 2014 and tilled with a Howard Rotavator (Germany) on 31 July 2014. On 20 August 2014, the field was cultivated twice with a field cultivator and soil finisher (John Deere, Ankeny, IA) for light cultivation and leveling. It was then cultipacked twice with a J. I. Case Cultipacker (Racine, Wisconsin) to firm up the seed bed before seeding.

Forty research plots (10 cultivars with four replications) were seeded 8 September 2014 in a randomized complete block, split-block, split-split plot in time, design. The 10 cultivars seeded represented four species: Kentucky bluegrass (*Poa pratensis* L.) 'Barderby' and 'Ginger', orchardgrass (*Dactylis glomerata* L.) 'Persist,' 'Profit' and 'Quickdraw', tall fescue (*Festuca arundinacea* Schreb.) 'Cajun II' and 'Bronson', and perennial ryegrass (*Lolium perenne* L.*)* 'Calibra,' 'Linn' and 'Aberzest'. These species were chosen as common cool-season grass species in central Kentucky horse pastures. For each species, cultivars estimated to have low or high in nonstructural carbohydrates were selected to represent the range of possible nonstructural carbohydrate concentrations within a species. Choices of high- and low- nonstructural carbohydrate cultivars were based on time to maturity (Olson et al., 2014a,b,c,d), with late-maturing cultivars expected to be highest in nonstructural carbohydrates (Jong-Duk Kim, Cheonan Yonam College, Cheonan City, South Korea, personal communication). For both perennial ryegrass and orchardgrass, one low and two high nonstructural carbohydrate cultivars were selected in case one of the high nonstructural carbohydrate cultivars had poor winter survival. The cultivars chosen for high- and low- nonstructural carbohydrates are listed in Table 3.1

Nitrogen treatments (0 and 157 kg N ha^{-1}) were applied as split-blocks to evaluate nonstructural carbohydrate variation from soil fertility, creating a total of 80 plots (Table 3.2). Nitrogen rate was based on University of Kentucky recommendations for typical Kentucky horse pastures (Schwab and Piersawl, 2010) (Table 3.3). The nitrogen treatments were applied as split applications: 56 kg N ha^{-1} (16 March 2015), 39 kg N ha^{-1} (13 May 2015), and 56 kg N ha^{-1} (19 August 2015). Applications were performed with a Gandy 3 ft drop-type fertilizer spreader (Owatonna, MN). For the May application of nitrogen, Agrotain (Koch Fertilizer, Wichita, KS) treated urea was used to reduce volatilization.

On 5 September 2014, soil samples were taken randomly across all replications and processed by University of Kentucky Regulatory Services. On 26 September 2014, fertility treatments were applied based on the University of Kentucky soil test recommendations (Schwab and Piersawl, 2010): 56 kg N ha^{-1} as urea ammonium nitrate, 23 kg K ha^{-1} as K_2O, and 3364 kg ha^{-1} pelletized lime. The plots were sprayed for broadleaf weed control on 29 September 2014 with 0.78 kg ha^{-1} 2,4-dichlorophenoxyacetic acid and 0.56 kg ha^{-1} dimethylamine salt of Dicamba. The plots were mowed at 12.5 cm forage height with a Kubota ZG227 zero turn mower (Torrance, CA) on 10 November 2014. On 23 July 2015, the plots were sprayed with 0.693 kg ha^{-1} Pendimethalin as a pre-emergent to control warm-season grasses.

Forage Sampling Procedures

Sampling began on 13 May 2015 when plots reached an average height of 15 to 20 cm and continued until 3 November 2015. Plots were sampled twice daily at 8:00-9:00 am and 3:00-4:00 pm. Forage height was measured at three random locations within each plot. Harvests occurred every two weeks, with the exception of 22 July 2015 to 13 October 2015, when four-week intervals were required to reach the desired regrowth height. Ten total harvests were performed from May to November (Table 3.4).

Plots were harvested in order of replication. Morning and afternoon harvests were sampled in the same order to stay consistent with the amount of time between morning and evening sampling for each plot. This study was unique in that forage maturity was maintained at the vegetative stage throughout the study. The exception was perennial ryegrass, which contained scattered seedheads from 13 May 2015 to 24 June 2015. The plots were mowed immediately after each sampling with a Kubota ZG227 zero turn mower to a height of 10 cm, in order to maintain a vegetative growth stage. Clippings were returned to the plots to represent typical horse pasture management.

Samples were collected by cutting approximately 100 g of forage at 5 cm forage height to mimic grazing. To ensure consistency, polyvinyl chloride (PVC) pipes 5 cm in diameter were positioned next to the forage being sampled, garden shears were placed on

top, and a handful of grass was sampled. Forage was then diced with garden shears to 2.5 to 5 cm length before being placed in a 20 cm diameter aluminum collecting pan. This procedure was repeated at five to ten random locations throughout each plot, until approximately 100 g of forage had been collected. The samples were immediately flash frozen in the field with liquid nitrogen by placing the pan in Styrofoam coolers that held the liquid nitrogen, so that there was enough liquid nitrogen to cover the bottom and sides of the collecting pan. Samples were transported in coolers with dry ice to -20 ^{o}C freezers where they were stored until they could be freeze dried. Samples were freeze dried for seven to ten days from -29 ^{o}C to 23 ^{o}C. Three freeze dryers were used to accommodate the large number of samples: Botanique (Phoenix, AZ) model 18DX48SA, VirTis SP Industries (Gardiner, NY) model 36X66GPFD, and Botanique (Phoenix, AZ) model 24DX54. Once samples were freeze dried, they were ground using a 1mm mesh in a Udy Cyclone Sample Mill (UDY Corporation, Fort Collins, CO) and scanned with a Foss 6500 Near Infrared Reflectance Spectroscopy (NIRS) (Foss, Inc., Hillderod, Denmark) with ISI software (Infrasoft International, L.L.C., State College, PA).

All cultivars were sampled at each harvest date with the exception of one plot (perennial ryegrass ‘Aberzest’ with nitrogen treatment) that became contaminated with another species during establishment, and therefore was not included in sampling and labeled as missing data for statistical analysis. Incomplete freeze drying also resulted in the loss of a set of samples: 1.75 out of 4 replicates of the morning sampling of harvest 3 (15 May 2015), and 4 samples from harvest 4 (27 May 2015). For these harvests, there were still enough samples to represent each treatment, and therefore the remaining harvest data were included in the analysis.

NIRS Sample Selection, Laboratory Procedures, NIRS Equation Calibration

Refer to Chapter 2: Predicting Water-soluble Carbohydrates and Ethanol-soluble Carbohydrates in Cool-season Grasses with Near Infrared Reflectance Spectroscopy, for information on NIRS sample selection, laboratory procedures, and NIRS equation calibration.

Weather Data Collection

The University of Kentucky North Farm weather station recorded weather data every 15 seconds and reported minimum, maximum, and means for every 15 minutes from 7 May 2015 through 19 November 2015. A minimum, maximum and mean reading was recorded for air temperature and photosynthetically active radiation (PAR). Total rain was also recorded. Average air temperature, total rain, and cumulative maximum PAR were used for the morning (12:00 am – 8:00 am) and afternoon (8:00 am – 3:00 pm) of each harvest date (Table 3.6) to support interpretation of results.

Statistical Analysis

Data were analyzed using SAS 9.3 (Cary, NC) PROC GLIMMIX procedures as a randomized complete block, split-block, split-split plot in time experimental design. Cultivar (10), time of day (2), harvest date (10), and fertility treatment (2) were treated as fixed effects, with block (4) treated as a random effect. Fertility treatment served as the split-block. Because the split-split plot in time was really a combination of two effects, harvest date by time of day interaction was used as the split-split plot in time and treated as a repeated measure. Heterogeneous compound symmetry (CSH) covariance structure was used. A total of 1531 samples from 2015 harvests were predicted by the NIRS and analyzed. Estimate contrasts were run on species for both WSC and ESC. All interactions were sorted by harvest date and therefore analyzed separately for each harvest date. For some interactions, the “slice” command in SAS 9.3 was also used to analyze individual effects within an interaction.

Table 3.1 The cool-season perennial grass cultivars seeded 8 September 2014 at University of Kentucky' North Farm selected for nonstructural carbohydrate research.

Species	**Cultivars predicted as low in nonstructural carbohydrate (early maturing)**	**Cultivars predicted as high in nonstructural carbohydrate (late maturing)**
Kentucky bluegrass	Ginger	Barderby
Tall fescue	Cajun II	Bronson
Orchardgrass	Persist	Profit and Quickdraw
Perennial ryegrass	Linn	Aberzest and Calibra

Table 3.2 Experimental design of 2015 nonstructural carbohydrate study at University of Kentucky's North Farm in Lexington, Kentucky. (TF=Tall Fescue, OG=Orchardgrass, BG=KY Bluegrass, PR=Perennial Ryegrass).

None	N		N	None		None	N		N	None	
101	201	TF Bronson	301	401	BG Barderby	501	601	OG Quickdraw	701	801	PR Linn
102	202	TF Cajun II	302	402	OG Persist	502	602	PR Linn	702	802	PR Calibra
103	203	OG Profit	303	403	BG Ginger	503	603	PR Aberzest	703	803	TF Bronson
104	204	OG Persist	304	404	PR Calibra	504	604	TF Cajun II	704	804	BG Barderby
105	205	OG Quickdraw	305	405	OG Profit	505	605	OG Persist	705	805	TF Cajun II
106	206	BG Ginger	306	406	PR Linn	506	606	PR Calibra	706	806	BG Ginger
107	207	BG Barderby	307	407	TF Cajun II	507	607	TF Bronson	707	807	OG Profit
108	208	PR Linn	308	408	PR Aberzest	508	608	BG Ginger	708	808	PR Aberzest
109	209	PR Calibra	309	409	TF Bronson	509	609	BG Barderby	709	809	OG Persist
110	210	PR Aberzest	310	410	OG Quickdraw	510	610	OG Profit	710	810	OG Quickdraw

Table 3.3 Recommended and actual nitrogen application rates on cool-season horse pastures when maintained at high stocking rates (less than 0.80 ha/horse) (Schwab and Piersawl, 2010).

Recommended Date	**Recommended nitrogen per application (kg ha^{-1})**	**Date applied**	**Nitrogen applied (kg ha^{-1})**
Feb. 15 – Mar. 15	up to 45 - 90	16 March 2015	56
May 1-15	up to 34 - 45	13 May 2015	39
Aug. 15-30	up to 45 - 90	19 August 2015	56

Table 3.4 Harvest dates for sampling ten cool-season grass cultivars measuring water-soluble carbohydrates and ethanol-soluble carbohydrates during the 2015 growing season in central Kentucky (n=160 per harvest), sampled in the morning from 8:00-9:00 and in the afternoon from 3:00-4:00.

Harvest #	**Date**
1	May 13
2	May 27
3	June 10
4	June 24
5	July 8
6	July 22
7	August 19
8	September 15
9	October 13
10	November 3

3.4 Results and Discussion

Species Effects on WSC and ESC

The species contrasts encompass all harvests from 13 May to 3 November 2015 and all nitrogen treatments. For WSC, all four species were significantly different from each other, with perennial ryegrass being the highest WSC accumulating cultivar at 8.18%, followed by tall fescue at 7.49%, KY bluegrass at 7.14%, and orchardgrass at 5.60% (Figure 3.1).

The species contrast for ESC followed similar patterns as those observed for WSC, except that there was no difference between perennial ryegrass and tall fescue (Figure 3.2). Since there was a difference in WSC between perennial ryegrass and tall fescue but no difference in ESC between the two species, this discrepancy may suggest that perennial ryegrass contains more long chain fructans than tall fescue. The ESC concentrations for tall fescue, perennial ryegrass, KY bluegrass and orchardgrass were 6.93%, 6.75%, 6.07%, and 4.98%, respectively. Overall, the concentrations for ESC of each species were lower than that of WSC, which is expected since ESC does not contain long chain fructans (Pavis et al., 2001)

These results are consistent with previous studies finding that tall fescue and perennial ryegrass accumulated some of the highest concentrations of nonstructural carbohydrates and orchardgrass some of the lowest. For example, Jensen et al. (2014) tested 17 species in an irrigated pasture in northern Utah and determined that perennial ryegrass contained the highest concentrations of WSC. Another study by Chatterton et al. (1989) compared TNC of 128 cool-season grass species, including perennial ryegrass, tall fescue and orchardgrass, and found that, among those three, tall fescue was the highest TNC species, followed by perennial ryegrass and then orchardgrass.

For horses at risk for laminitis or other metabolic conditions, it is recommended that the total TNC content of their diet be less than 10% on a dry matter basis (Frank et al., 2010b). The present study measured WSC, but starch would need to be added to calculate TNC, even though concentrations are usually considered minimal in cool-season grass species. All four species WSC means across all harvest dates are below 10%, with the

highest WSC accumulating species, perennial ryegrass, having a mean of 8.18%. These values are also much lower than those used in the Pollitt and Milinovich (2017) article discussed in Chapter 1, so horses would need to consume a considerable amount of the present study's forage to compare with those values. When looking at specific harvest dates, however, several cultivars had WSC means considerably higher than 10%, particularly in the spring and fall months, which will be discussed in depth further on (Table 3.5A and 3.5B).

Water-soluble Carbohydrate Interactions

There were three significant three-way interactions for WSC: cultivar by fertility by harvest date ($p<0.0001$), cultivar by harvest date by time of day ($p<0.0001$), and fertility by harvest date by time of day ($p=0.0030$). All three interactions were sorted by harvest date and therefore analyzed separately for each harvest date.

For ease of interpretation, the highest and lowest WSC or ESC "group" was assigned to results for each harvest date. In tables 3.5, 3.7, 3.8, and 3.9, the highest group is designed with a red background, and the lowest with a yellow background. Groups were assigned based on letter groupings determined from the lsd test. For example, for each harvest the highest group would all have a letter A, and the lowest group would all have the last letter option of that harvest date.

Cultivar by Harvest Date by Time of Day Response for WSC

The perennial ryegrass cultivar Aberzest in the afternoon was in the highest WSC group for the cultivar/time of day combinations across all ten harvests (Table 3.5A and 3.5B). WSC for Aberzest ranged from a high of 15.18% on the afternoon of 13 May to a low of 5.48% on the morning of 8 July. Aberzest am was in the highest WSC group on 27 May and 15 September, and Calibra was in the highest WSC group on 13 May (am and pm) and 3 November pm. The afternoon samples of tall fescue cultivars Bronson and Cajun II were in the highest WSC group of a few harvests. Specifically, the Bronson pm samples

were in the group accumulating the most WSC on 22 July, 15 September and 3 November, and Cajun II pm samples were in the group accumulating the most WSC on 15 September and 3 November. KY bluegrass Ginger pm was also in the highest WSC group on 19 August and 3 November.

For all ten harvests, the three orchardgrass cultivars were in the lowest WSC group in the morning, with the exception of Persist am on 13 May (Table 3.5A and 3.5B). On 8 July, orchardgrass Persist pm was also in the lowest WSC group, as was KY bluegrass Barderby am on 8 July, 13 October and 3 November. On 15 September, Barderby am, Ginger am, Persist pm, Profit pm, and Quickdraw pm were in the group lowest in WSC. On 15 September there were no differences between am and pm WSC concentrations for the majority of cultivars. This lack of difference may have been due to weather conditions. From midnight to 8:00 am on 15 September, the average air temperature (12 ^{o}C) was among the lowest air temperatures up to that point in the growing season (Table 3.6). Cooler nighttime temperatures could result in decreased respiration rates and relatively high morning WSC concentrations in the morning. For the afternoon sampling, the cumulative maximum PAR from 8:00 am to 3:00 pm was relatively low on this date (23,594 umol/s/m^2) compared to previous harvest dates. This decreased light intensity during the day could result in less nonstructural carbohydrate production during the day, decreasing afternoon WSC concentrations. The average air temperature from 8:00 am to 3:00 pm was also high enough (21 ^{o}C) that respiration would be using nonstructural carbohydrates during the day. The combination of cool nighttime temperatures, high daytime temperatures and low PAR could result in the morning and afternoon WSC concentrations being closer together than on other harvest dates. Kagan et al. (2011) saw similar results in that similar WSC concentrations were observed for both morning and afternoon samples of one sampling day. They observed low PAR prior to the sampling day, and suggested that the low PAR led to lower than usual afternoon concentrations.

The results from this research are supported by previous studies that nonstructural carbohydrate concentrations are typically higher in the afternoon than in the morning (Lechtenburg et al., 1972; Ciavarella et al., 2000; Shewmaker et al., 2006; Kagan et al., 2011). Since carbohydrates are produced through photosynthesis and utilized through

respiration during the day, and at night photosynthesis ceases but respiration is continued (Watts and Chatterton, 2004), it is expected that carbohydrate concentrations will be lowest in the morning and highest in the afternoon.

Fertility by Harvest Date by Time of Day Response for WSC

For all ten harvests, the afternoon samples accumulated the highest WSC concentrations across cultivars, but there was no difference between nitrogen and no nitrogen on most harvest dates (Figure 3.3). These results suggest that WSC concentrations will be high in the afternoon regardless of nitrogen applications, so nitrogen treatments may not be a means to lower peak WSC concentrations. The only exception to this trend was 8 July, where afternoon WSC was lower in the presence (4.81%) than in the absence (5.14%) of nitrogen. This difference was expected, because nitrogen applications have been reported to decrease WSC concentrations (Brown and Blaser, 1965; Lechtenburg et al., 1972), but it was surprising that it did not occur more often. An abundance of nitrogen should stimulate growth in the plant, requiring the use of nonstructural carbohydrates, resulting in fewer reserve carbohydrates. Interestingly, on 8 July, the concentrations in the afternoon were lower on a numerical basis than on other harvest dates in the afternoon. This may be explained by the extremely low (8535 umol/s/m^2) cumulative maximum PAR from 8:00 am to 3:00 pm on 8 July, the lowest daytime PAR for all harvest dates (Table 3.6). In addition, the average air temperature during that time (23 ^oC) was among one of the highest daytime temperatures across harvest dates (Table 3.6). The combination of low PAR and high temperatures during the day likely resulted in the lower overall WSC concentrations when compared to other harvest date concentrations in the afternoon.

Overall, the morning WSC concentrations across cultivars were consistently lower than both nitrogen treatments (with and without) in the afternoon. For the majority of harvests, (13 May, 10 June, 24 June, 8 July, 19 August, and 15 September), there was also no difference in nitrogen treatments for the morning samples, indicating that the nitrogen treatments had no effect. The exceptions were 27 May, 13 Oct, and 3 Nov, where nitrogen plots in the morning contained less WSC than those without nitrogen, an expected trend. July 22 was the only harvest date exhibiting higher morning WSC with than without

nitrogen. This harvest date had an average air temperature of 19 ^{o}C from midnight to 8:00 am that morning, and a cumulative maximum PAR of 797 umol/s/m^2 during that period (Table 3.6), which is normal for that time of year in central Kentucky. Sprague and Sullivan (1950) found fructan concentrations to be higher in low-nitrogen treated plants than in high-nitrogen treated plants, but in their study the results for other individual sugars were variable. The results from this present study may indicate that the nitrogen treatment was low enough to cause fructan to accumulate on the morning of this harvest date, but not high enough to stimulate utilization of reserve carbohydrates.

Also, this study had a frequent mowing regime of every two to four weeks. Defoliation has been shown to decrease sucrose and fructan (Sprague and Sullivan, 1950), and WSC (Lacey et al., 1994) concentrations by stimulating growth and utilizing reserve carbohydrates. Sprague and Sullivan (1950) found that after the immediate drop in sucrose and fructan concentrations, initial concentrations were restored before the next cutting 35 days later. Since the present study involved mowing every 14 or 28 days, WSC concentrations were likely decreased again before initial concentrations were restored, resulting in lower overall WSC concentrations. Jensen et al. (2014) had longer defoliation intervals, harvesting the whole plot once in August during the sampling period of May to November. The WSC concentrations in that study for cool-season grasses ranged from 5% (orchardgrass on 3 August) to 29% (perennial ryegrass on 2 June). These concentrations are much higher than those in the present study, where WSC concentrations typically stayed between 3% and 15%, with species WSC means closer to 6-8%. Therefore, the frequent mowing intervals of the present study may have impacted overall WSC concentrations and should be considered in pasture management.

Given the number of instances in which afternoon WSC exceeded morning WSC, if a horse manager desires a diet with minimal nonstructural carbohydrates, then grazing in the morning in contrast to the afternoon makes sense. Frank et al. (2010b) recommended that at risk horses should be fed a diet with less than 10% TNC on a dry matter basis. Since the present study did not measure starch, starch would need to be added to compare the WSC concentrations to TNC. It is likely that, even with the addition of starch, the morning WSC concentrations would still fall below 10%, especially if orchardgrass or Kentucky

bluegrass are being grazed. Perennial ryegrass and tall fescue, however, still had some high morning WSC concentrations on some harvest dates, particularly 13 May, 15 September, and 3 November. In the afternoon, many WSC concentrations were close to or above 10%. The perennial ryegrass cultivar Aberzest reached 15% on the afternoon of 13 May. The highest concentrations occurred in the spring and fall, especially with perennial ryegrass and tall fescue. Therefore, species and seasonal variation should also be considered in addition to time of day.

While WSC concentrations will vary throughout the season and some afternoon concentrations may be lower than others, this study has shown that morning concentrations are typically lower than afternoon concentrations. Grazing in the morning may or may not be practical depending on the resources of the facility and logistics of the operation. For horses that are typically turned out 24/7, limiting grazing to the morning may not be feasible. Farms that operate on a daily turnout schedule will have more flexibility of turning out early in the morning and bringing in in the afternoon before peak nonstructural carbohydrate concentrations are reached. Many horse farms are already on this schedule in the winter months, where turnout is utilized during the day and horses stay up at night. Horses could then be brought in late morning before concentrations substantially increase. This rotation would work best in the summer months when many operations utilize night turnout to avoid summer heat. Also, as stated above, mowing has been shown to reduce WSC concentrations (Sprague and Sullivan, 1950; Lacey et al., 1994), so adopting a frequent mowing regime may help decrease WSC concentrations.

In contrast, if a dairy or beef cattle farm desires a high nonstructural carbohydrate diet, grazing should occur in the afternoon. Again, this grazing rotation may or may not be practical depending on the operation. For dairy operations where cattle have to be brought in twice daily for milking, late afternoon grazing following afternoon milking is feasible. For beef cattle managers, afternoon grazing would work best with an intensive rotational grazing system. Since mowing has been known to decrease WSC concentrations (Sprague and Sullivan, 1950; Lacey et al., 1994), limiting mowing would be best to conserve nonstructural carbohydrates. However, limiting mowing would also allow the plant to

increase in maturity, and there is a drop in forage quality as plants mature (Ball et al., 2001), so this compromise should be considered in management decisions.

Cultivar by Fertility by Harvest Date Response for WSC

For all harvests, perennial ryegrass Aberzest without nitrogen was in the highest WSC group for cultivar/fertility combinations (Table 3.7A and 3.7B). In general, perennial ryegrass entries, specifically Aberzest, were among the highest WSC accumulating cultivars across all harvests. Aberzest without nitrogen ranged from 5.99% WSC to 14.50% WSC throughout the growing season.

For all harvests except for 27 May, 13 October, and 3 November, Aberzest with nitrogen did not differ from Aberzest without nitrogen. On 13 May, there were no differences between Aberzest without nitrogen, Aberzest with nitrogen, perennial ryegrass Calibra without nitrogen, and Calibra with nitrogen. (Note: at this point only one treatment of 56 kg N ha^{-1} had been applied to the split plots). Tall fescue Bronson with nitrogen was also in the highest WSC group on 22 July and 15 September, with no differences from Aberzest with and without nitrogen. These results demonstrated that Bronson accumulated relatively high amounts of WSC at this time of year. On 3 November, perennial ryegrass Calibra without nitrogen did not differ from Aberzest without nitrogen.

All orchardgrass cultivars, both with and without nitrogen, were in the group accumulating the least WSC in eight out of the ten total harvests (Table 3.7A and 3.7B). Quickdraw with nitrogen was in the lowest WSC group for all ten harvests. Quickdraw without nitrogen and Profit without nitrogen were in the lowest WSC group for nine out of ten harvests. Profit with nitrogen, Persist with nitrogen, and Persist without nitrogen were in the lowest WSC group for eight out of ten total harvests. In addition, KY bluegrass Barderby without nitrogen was in the lowest WSC group from 15 September to 3 November, and KY bluegrass Barderby with nitrogen was the lowest WSC group for 15 September. These results suggest that nitrogen treatments may not have a consistent effect on WSC. Other factors, such as species and time of day, may have greater effects on WSC concentrations.

Horse managers who desire a diet with minimal nonstructural carbohydrates should utilize a low nonstructural carbohydrate accumulating cultivar. These results suggest that in late fall, KY bluegrass Barderby accumulates low WSC concentrations. Also, all three orchardgrass cultivars, Persist, Profit and Quickdraw, were consistently the lowest WSC accumulating cultivars, and all three cultivars were comparable in WSC concentrations. It is important to remember that while orchardgrass cultivars were comparable in WSC in this study, WSC concentrations for specific cultivars may vary. For example, Kagan et al. 2011 observed relatively high WSC concentrations in the spring of orchardgrass 'Potomac'. They observed WSC concentrations of around 12% and 15% for morning and afternoon samples of 12 May, respectively. These concentrations are quite high when compared to the present study where mean WSC concentrations were around 6% and 8% for morning and afternoon orchardgrass samples on 13 May. This discrepancy could be due to a few different reasons. Even though the present study saw no differences between cultivars of orchardgrass, Potomac was not studied so there is the potential that this is a higher WSC cultivar. Also, the Kagan et al. 2011 study used a different WSC assay method than the present study, a potassium ferricyanide colorimetric assay, which could have an effect on final values. Another factor to consider is climate; the Kagan et al. 2011 study was done in Piedmont region of Virginia, and environmental factors can have a large impact on nonstructural carbohydrate concentrations.

Beef or dairy managers who desire a high nonstructural carbohydrate diet should utilize a high nonstructural carbohydrate accumulating cultivar. Overall, perennial ryegrass Aberzest had the highest WSC concentration across all ten harvest dates. Calibra also had high WSC concentrations in early spring and late fall. KY bluegrass Ginger and tall fescue Bronson and Cajun II also accumulated high WSC concentrations during the afternoon in the fall. Other factors need to be considered when choosing a new cultivar to seed. For example, in central Kentucky, the average stand life of perennial ryegrass is two years (Olson et al., 2014d), and longevity is an important factor for overall economic value. Tall fescue may be another good option as it exhibits greater persistence in Kentucky (Olson et al., 2014b; Olson et al., 2014e) and still has fairly high nonstructural carbohydrate concentrations when compared to other species. Only two tall fescue cultivars, Bronson

and Cajun II, were tested in this study, but it is likely that other tall fescue cultivars would provide similar results (Shewmaker et al., 2006).

Cultivar by Fertility by Harvest Date Response for WSC, Separated by Fertility and Cultivar

Interactions were separated by fertility and cultivar to evaluate differences in WSC from individual effects (Table 3.7A and 3.7B). When separated by fertility treatments, both nitrogen treatments were significant ($p<0.0001$), meaning that for both with and without nitrogen, there were significant cultivar differences due to the large range of WSC accumulation in cultivars. However, when separated by cultivar, the differences varied for each harvest.

On 13 May, which was right before the second spring nitrogen application, there were no cultivar differences in WSC concentrations of different fertility treatments except for orchardgrass Profit, where WSC was higher in the plots with nitrogen than without nitrogen (at this point only one of the two spring nitrogen treatments had been applied). On 27 May, two weeks after the spring nitrogen application, all KY bluegrass cultivars and all perennial ryegrass cultivars differed in WSC concentrations between fertility treatments, with the nitrogen treatment accumulating less WSC than no nitrogen. Since nitrogen stimulates growth and utilizes nonstructural carbohydrates for growth (Jacobs et al., 1989), it was predicted that the nitrogen treated plots would be lower in WSC than those without nitrogen. Tall fescue and orchardgrass cultivars, however, did not differ in WSC based on fertility on this date. This result suggests that different cool-season grass species may respond differently to nitrogen treatments.

The 10 June harvest was four weeks after the second nitrogen treatment of 39 kg N ha^{-1}, and the only cultivars differing in WSC between fertility treatments were perennial ryegrass Calibra and tall fescue Bronson and Cajun II. For Calibra, WSC was lower in the nitrogen plots, as expected from other studies (Lechtenberg et al., 1972; Jacobs et al., 1989). The tall fescue cultivars, however, had higher WSC concentrations in the nitrogen treated plots. These data demonstrated an inconsistency among cultivars in effects of

nitrogen treatments on WSC accumulation, suggesting that different cultivars may respond differently to fertility treatments, and that, in this study, overall, fertility plays less of a role in determining WSC concentrations than other factors such as genotype and environment.

Six weeks following the second nitrogen application, 24 June, there were no within-cultivar differences between fertility treatments except for orchardgrass Persist, which had less WSC in the nitrogen treated plots. No differences between fertility treatments were found in cultivars based on fertility treatment on 8 July 2015, 19 August and 15 September, though KY bluegrass Barderby showed higher WSC in the nitrogen plots on 22 July.

The last nitrogen treatment of 56 kg N ha-1 was applied on 19 August. Eight weeks after this application, 13 October, the only cultivars exhibiting differences in WSC concentrations between fertility treatments were KY bluegrass Barderby, and perennial ryegrass Aberzest and Calibra. Perennial ryegrass Aberzest and Calibra accumulated less WSC with nitrogen, whereas KY bluegrass Barderby accumulated more WSC with nitrogen than without nitrogen. Eleven weeks after the last nitrogen application, on 3 November, the same trends as on 13 October were observed. In addition, perennial ryegrass Linn accumulated less WSC with nitrogen.

To evaluate effects of fertility treatment on different cultivars, the cultivar by fertility treatment by harvest date interaction was used. These results show that shortly following nitrogen application, decreased WSC was observed in some species, with perennial ryegrass and KY bluegrass accumulating less WSC in the nitrogen treated plots than the plots without nitrogen. Several weeks following nitrogen treatments (8 July), no cultivars differed in WSC between fertility treatments, suggesting that nitrogen effects on WSC may decrease rapidly, having less of an impact on WSC than genetic or environmental factors. Furthermore, results for some cultivars in subsequent harvests contradicted expected trends, with higher WSC in nitrogen treated plots in than the untreated plots. Although the WSC response to nitrogen was variable in this study, most previous research shows that nitrogen applications decrease WSC concentrations in response to increased forage growth. Lechtenburg et al. (1971) observed lower fructan and Jacobs et al. (1989) observed lower overall WSC after nitrogen applications. Brown and Blaser (1965) reported that under rapid grass growth conditions including ideal nitrogen,

temperature and rainfall, carbohydrates remained at low levels or decreased, while conditions reducing growth resulted in accumulation of carbohydrates. Sprague and Sullivan (1950) reported that fructan and sucrose were lower in orchardgrass under high nitrogen applications when compared to plants under low nitrogen applications. High nitrogen stimulates growth more so than low nitrogen, so more reserve carbohydrates would be used. Their study also suggests that utilization of simple sugars occurs only when simple sugar concentrations are high, but not at lower concentrations. Sucrose utilization, in particular, was greater when initial sucrose concentrations were high (Sprague and Sullivan, 1950).

In summary, the response of applied nitrogen to WSC in this study varied with harvest date and cultivar, and therefore would not be recommended as a reliable tool to manipulate nonstructural carbohydrate concentrations. If the goal is to create a low nonstructural carbohydrate pasture, it may be an advantage to utilize grazing immediately following nitrogen application. However, unless the pasture is perennial ryegrass or KY bluegrass, WSC concentrations may not decrease following nitrogen applications. If the goal is to create a high nonstructural carbohydrate pasture, nitrogen treatments would not be recommended to change nonstructural carbohydrate concentrations, due to the inconsistency across cultivars and harvest dates. Also, the yield lost by not utilizing nitrogen would likely not be worth any potential increase in nonstructural carbohydrate concentration. Mowing regime would also likely have an impact on WSC concentrations as defoliation has been known to decrease WSC concentrations (Sprague and Sullivan, 1950; Lacey et al., 1994).

Ethanol-soluble Carbohydrate Interactions

Two significant three-way interactions were observed for ethanol-soluble carbohydrates: cultivar by fertility treatment by harvest date ($p<0.0001$) and cultivar by harvest date by time of day ($p<0.0001$). Both interactions were sorted by harvest date and therefore analyzed by each individual harvest.

Cultivar by Harvest Date by Time of Day Response for ESC

The cultivar by harvest date by time of day interaction for ESC was significant at $p<0.0001$ (Table 3.8A and 3.8B). This interaction was separated by cultivar and time of day to evaluate individual effects. When separating by time of day, both am and pm ESC concentrations differed between cultivars ($p<0.0001$) at each harvest date. In other words, in both the morning and afternoon, significant effects due to cultivar were present, likely due to the wide range of cultivars in the study.

Separating by cultivar showed that for most cultivars on most harvest dates, the afternoon ESC concentrations were significantly higher than the morning concentrations. The only exception was on 15 September, in which there was no difference between morning and afternoon ESC concentrations for KY bluegrass Barderby, perennial ryegrass Linn, and all three orchardgrass cultivars. The lack of difference in am and pm ESC on 15 September may have been due to weather conditions (Table 3.6). As discussed with WSC, cooler nighttime temperatures (12 ^{o}C) could result in decreased respiration rates and relatively high morning ESC concentrations in the morning. The cumulative maximum PAR during the day was relatively low (23,594 umol/s/m^2) and the average air temperature during the day was high enough (21 ^{o}C) that respiration would be using nonstructural carbohydrates during the day. The combination of cool nighttime temperatures, high daytime temperatures and low PAR could result in the morning and afternoon ESC concentrations being closer together.

Overall, the samples highest in ESC were the afternoon samples of perennial ryegrass Aberzest and Calibra, and both tall fescue cultivars in the afternoon (Table 3.8A and 3.8B). The highest ESC accumulating cultivar differed among harvests, but these four cultivars were in the highest ESC group on at least four harvest dates. Aberzest in the afternoon was in the highest ESC group for eight out of the ten total harvests, and Calibra in the afternoon was in the highest ESC group for the first two harvests and last two harvests (13 May, 27 May, 13 October and 3 November). Tall fescue Bronson in the afternoon was in the highest ESC group for seven out of ten total harvests, and Cajun II in the afternoon was in the highest ESC group for five out of the ten total harvests. Additionally, two other cultivars were among the highest ESC accumulating cultivars for

one harvest each: KY bluegrass Barderby pm on 19 August, and perennial ryegrass Linn pm on 13 May.

Overall, the lowest ESC cultivar/time of day combinations across all harvests were the morning samples for all three orchardgrass cultivars (Table 3.8A and 3.8B). Each of these am cultivars was in the lowest ESC group for at least eight of the ten total harvests. Similarly to the findings for WSC, on 15 September the three orchardgrass cultivars did not differ in am and pm ESC concentrations. All had the lowest ESC concentrations for that harvest date. As stated previously, 15 September had cool nighttime temperatures, high daytime temperatures and low PAR that could have resulted in the morning and afternoon ESC concentrations being closer together (Table 3.6). Perennial ryegrass Linn am was also in the lowest ESC group on that date, as well as on 19 August. Additionally, KY bluegrass Barderby am was in the lowest ESC group on 13 October and 3 November.

Cultivar by Fertility by Harvest Date Response for ESC

Several cultivar/fertility combinations were in the group highest in ESC throughout the growing season (Table 3.9A and 3.9B). Overall, perennial ryegrass Aberzest with and without nitrogen, tall fescue Bronson with nitrogen and tall fescue Cajun II with nitrogen were among the highest ESC accumulators, each in the highest ESC group on at least six of the ten total harvests. Cultivar/fertility combinations in the group highest in ESC for one or more harvests were KY bluegrass Barderby with and without nitrogen on 8 July and 19 August, and KY bluegrass Ginger with nitrogen on 24 June and 8 July. Others were perennial ryegrass Calibra with and without nitrogen on 13 May and 27 May, perennial ryegrass Linn with and without nitrogen on 13 May, tall fescue Bronson without nitrogen on 24 June, 8 July, and 15 September, and tall fescue Cajun II without nitrogen on 24 June, 8 July, and 15 September.

Comparing high ESC accumulating cultivars to high WSC accumulating cultivars revealed that they were similar, in that perennial ryegrass Aberzest had the highest concentrations of ESC and WSC across most harvest dates. However, Bronson and Cajun II were high in ESC beginning on 27 May 2015 and continuing periodically throughout the

season, but were not among the highest WSC accumulators on those harvest dates. One possibility is that tall fescue cultivars do not produce as many long chain fructans early in the growing season. Shewmaker et al. (2006) documented changes in the proportion of fructan, sucrose, glucose and starch in TNC for eight tall fescue cultivars on four sampling dates from mid-May to mid-September. They found that the relative amounts of different classes of carbohydrates varied among cultivars. Furthermore, the percentage of fructan in TNC for tall fescue was highest in July, but sucrose was the largest contributor to TNC for the rest of the sampling dates.

The lowest ESC cultivar/fertility combinations were the three orchardgrass cultivars without nitrogen for at least seven of the ten total harvests (Table 3.9A and 3.9B). Persist without nitrogen was in the lowest group for all harvests with the exception of 13 May, 24 June, and 3 November. Profit without nitrogen was in the lowest group for all ten harvests, and Quickdraw without nitrogen was in the lowest group for all harvests except for 22 July. KY bluegrass Barderby without nitrogen was also in the lowest ESC group on 3 November, as was perennial ryegrass Linn without nitrogen on 10 June and 15 September.

In summary, this study observed that while the nitrogen treatments were associated with some low ESC concentrations, the plots without nitrogen actually had lower ESC concentrations on more harvest dates. These results contradict expected outcomes that nitrogen would stimulate growth, utilizing nonstructural carbohydrates and decreasing concentrations (Brown and Blaser, 1965). Sprague and Sullivan (1950), however, saw similar results to the present study in that the application of high rates of nitrogen caused significant increases in reducing sugars (ESC) a few weeks later. While reducing sugars increased with nitrogen application, fructan decreased under low nitrogen rates in this study (Sprague and Sullivan, 1950). This difference suggests that specific sugars respond differently to nitrogen and may explain why the present study showed different WSC and ESC responses to nitrogen.

Also, the present study involved mowing the research plots every two to four weeks. Defoliation stimulates grass growth, utilizing nonstructural carbohydrates and decreasing overall concentrations. Studies have found that defoliation decreases ESC

(Sprague and Sullivan, 1950). Therefore, frequent mowing intervals may have had an impact on overall ESC concentrations and should be considered in pasture management.

Comparing low ESC cultivars to low WSC cultivars reveals similarities in that the three orchardgrass cultivars were in the lowest group across most harvest dates, and the KY bluegrass Barderby on fall mornings also had low concentrations. The morning samples of perennial ryegrass Linn were low on 19 August 2015 and 15 September 2015 for ESC but not for WSC, indicating possible variance in long chain fructan distribution throughout the growing season. Jensen et al. (2014) observed large variations in fructan percentage among species, with perennial ryegrass, and KY bluegrass containing the largest percentage of fructan in WSC, at 54%, and 42%, respectively. Shewmaker et al. (2006) also observed variation in carbohydrate fractions of several tall fescue cultivars.

Cultivar by Fertility by Harvest Date Response for ESC, Separated by Cultivar and Fertility

The cultivar by fertility treatment was separated by both factors to evaluate differences from individual effects (Table 3.9A and 3.9B). For all ten harvests, both nitrogen and no nitrogen were significant at $p<0.0001$, indicating that for each fertility level, there were significant differences in the cultivars due to the wide range of ESC concentrations that the different cultivars accumulated. When separated by fertility, as with WSC, some harvests exhibited differences between fertility treatments, and others did not.

On 13 May, right before the second nitrogen application, there were no differences in ESC between fertility treatments for any cultivar, with the exception of orchardgrass Profit and tall fescue Bronson. For Profit, ESC was higher in the presence than in the absence of nitrogen, while Bronson produced higher ESC without nitrogen than with nitrogen. On 27 May, two weeks following the second nitrogen application, the only cultivars differing between fertility treatments were the three orchardgrass cultivars, perennial ryegrass Linn, and both tall fescue cultivars. For all six of these cultivars, ESC was significantly higher in the nitrogen treated plots than in the plots without nitrogen.

On 10 June 2015, four weeks following the second nitrogen application, orchardgrass Quickdraw, perennial ryegrass Linn, and tall fescue Bronson and Cajun II continued to exhibit differences between fertility treatments, with nitrogen treated plots containing higher ESC than plots without nitrogen. On 24 June, six weeks following the second nitrogen application, the only cultivar differing in ESC between fertility treatments was orchardgrass Persist. However, on this date the samples without nitrogen contained more ESC than the samples with nitrogen. By 8 July, eight weeks following the second nitrogen application, there were no fertility treatment differences in ESC for any cultivar. On 22 July, both orchardgrass Persist and tall fescue Bronson with nitrogen contained significantly higher ESC than the corresponding plots without nitrogen. Perennial ryegrass Linn, however, accumulated more ESC with no nitrogen. There were no fertility differences for ESC on 19 August. On 15 September, the only cultivar exhibiting differences was perennial ryegrass Aberzest, with nitrogen plots having lower ESC than the plots without nitrogen.

On 13 October, eight weeks after the last nitrogen application, all cultivars showed a response to fertility except for KY bluegrass Barderby and perennial ryegrass Calibra. The remaining eight cultivars accumulated more ESC with than without nitrogen. By 3 November, 11 weeks after the final nitrogen application, all cultivars except perennial ryegrass Calibra, perennial ryegrass Linn and tall fescue Bronson differed in ESC between fertility treatments. These differences were similar to the previous harvest, with higher ESC concentrations in the presence than in the absence of nitrogen.

In summary, two weeks following the second nitrogen application, all three orchardgrass cultivars, perennial ryegrass Linn, and both tall fescue cultivars accumulated more ESC with nitrogen than without, contradicting expecting trends. These differences persisted in these cultivars, though slowly wearing off and showing no differences in cultivars from fertility by eight weeks after the second nitrogen application. There was little difference throughout the rest of the season until the last nitrogen treatment was applied. After the last treatment, however, a response to fertility was observed for most cultivars, with plots receiving nitrogen having higher ESC than those without nitrogen. This trend contradicts what was expected from the literature (Brown and Blaser, 1965) with the

addition of nitrogen. As discussed above, however, Sprague and Sullivan (1950) saw similar results to the present study in that, under high rates of nitrogen, reducing sugars (ESC) increased a few weeks following application. Their study suggests variability in effects on different classes of nonstructural carbohydrates, and may explain varying responses seen in the present study between ESC and WSC to nitrogen applications. Accumulation of ESC in response to applied nitrogen was inconsistent. Therefore, nitrogen application would not be recommended as a reliable tool to manipulate nonstructural carbohydrate concentrations, as effects of genotype and environment may play a greater role.

The frequent mowing regime used in this study should be considered when interpreting these results. Sprague and Sullivan (1950) took cuttings every 35 days, sampling on days 3, 7, 14, 21, 28 and 35, and saw variability in sucrose concentrations based on the time interval since last cutting. Particularly, they saw a drop in sucrose immediately following cutting (Sprague and Sullivan, 1950). The present study sampled every 14 or 28 days following cutting. If the present study was sampled more frequently and mowed less often, a larger range of ESC concentrations may have been observed.

3.5 Conclusions

In conclusion, the results from this research confirmed that water-soluble carbohydrate and ethanol-soluble carbohydrate concentrations are dependent on an interaction of factors including species, cultivar, fertility, time of day and harvest date. Significant species effects were observed for WSC, with highest to lowest concentrations in perennial ryegrass, tall fescue, KY bluegrass, and orchardgrass. ESC followed the same pattern but no difference was observed between perennial ryegrass and tall fescue. Cultivar effects were also observed, varying slightly with interaction of other factors, but with perennial ryegrass Aberzest being consistently one of the highest WSC and ESC accumulating cultivars, and all three orchardgrass cultivars Persist, Profit and Quickdraw consistently among the lowest WSC and ESC accumulating cultivars. The effects of nitrogen fertility were inconsistent across cultivars and harvest dates. Therefore, fertilization is not a reliable tool to manipulate WSC and ESC concentrations. Diurnal

effects followed a consistent pattern, with higher WSC and ESC concentrations consistently occurring in the afternoon and lower WSC and ESC concentrations occurring in the morning. The cumulative maximum PAR ranged from 12 to 911 umol/s/m^2 for the morning harvest dates and from 8,535 to 32,493 umol/s/m^2 for the afternoon harvest dates (Table 3.6), reflecting differences in morning and afternoon WSC and ESC.

3.6 Summary and Future Implications

In summary, diurnal effects on nonstructural carbohydrates of cool-season grasses were consistent with previous studies. Significant differences in nonstructural carbohydrates in the morning and afternoon are well documented in literature and generally confirmed by this study. Therefore, diurnal variance should be a main consideration in determining optimal grazing periods. Significant species differences were also presented and consistent with previous studies, with perennial ryegrass being the highest WSC and ESC accumulating species, followed by tall fescue, KY bluegrass, and orchardgrass. Nitrogen fertility effects, however, were inconsistent among harvest dates and cultivars, differing from other studies on some harvest dates, and therefore may not be a reliable indicator of nonstructural carbohydrate concentrations in pastures.

Recommendations for future research would be to repeat this study for an additional one or more years in another year/environment to validate results and collect more information. Diurnal and species differences may not change but there may be more definitive results for nonstructural carbohydrate response to nitrogen fertility. Also, additional years of weather data would provide more insight on the environmental effects on nonstructural carbohydrate concentration, and comparisons could be made across multiple years based on weather patterns. Methodology should be similar to this study, and analyzing individual sugars within WSC may provide more insight in interpreting the results. For example, comparing how fructan and sucrose change specifically throughout the season would aid in explaining why certain changes were observed in WSC.

These results show that more comprehensive studies are needed on nonstructural carbohydrate concentration response to nitrogen fertility. Another nitrogen rate could be

added so there are three nitrogen treatments: no nitrogen, a low rate of nitrogen, and a high rate of nitrogen. Adding another nitrogen rate is supported by Sprague and Sullivan (1950), who saw varying responses in sugar composition to low and high rates of nitrogen. If enough growth is available, weekly harvests could be performed following nitrogen applications to more closely monitor nonstructural carbohydrate changes over time. Comparing effects of less severe clipping regimes (this study mowed every two to four weeks) would also be useful to evaluate the role that different clipping intervals would play. Yield data would be a useful component in interpreting nitrogen responses. Measuring yield on short harvest intervals would require special machinery, so non-destructive yield measuring tools such as a rising plate meter may be a good option.

Since nonstructural carbohydrates fluctuate rapidly in pastures, having a simple method to quantify nonstructural carbohydrates would be useful to successfully manage grazing animals based on low or high nonstructural carbohydrate diets. Using only laboratory chemistry to quantify nonstructural carbohydrates is time consuming and costly. One objective of this study was to develop calibrated equations for faster and easier analysis of water-soluble carbohydrates (WSC) and ethanol-soluble carbohydrates (ESC) in cool-season grasses in Kentucky using near infrared reflectance spectroscopy (NIRS). The NIRS equations were developed so that they could be publicly available for future studies and eventually be a tool available to animal managers. Compared to relying entirely on wet chemistry, NIRS is a more economic and time-efficient method of measuring nonstructural carbohydrates in pastures. The NIRS equations for both WSC and ESC accurately predicted wet chemistry values for a wide range of values, from around 2% up to 20%. Both equations also had high 1-VR and R^2 values of over .90 and .93, respectively, indicating strong predictability. This method will aid in simple and timely monitoring of nonstructural carbohydrate levels in pastures in the future, and will allow for the more efficient management of grazing animals. The NIRS equations should be expanded with additional wet chemistry data as more data become available, especially over additional years and environments. Ideally, the calibration of a handheld NIRS would allow more rapid in-field monitoring of nonstructural carbohydrate concentrations.

Another important area of research is studying animal response to low and high nonstructural carbohydrate pastures. Laminitis has been induced experimentally by administering concentrations of starch that are high enough (Garner et al., 1977) to exceed enzymatic digestion in the small intestine and push undigested starch into the large intestine. Studies looking at changes in the horse directly from grazing pasture are also being performed and would be beneficial to continue pursuing. Frank et al. (2010a) observed positive correlations between insulin concentrations in horses [insulin resistance is a risk factor for laminitis in horses (Selim et al., 2015)] and ESC concentrations of the grass they were grazing. They also saw peaks of glucose and insulin concentrations in September when horses were grazing on pasture, which could contribute to the seasonal pattern of laminitis (Frank et al., 2010a). In their study, monthly mean ESC and WSC ranged from 2.0 to 9.1% and 1.6 to 12.7% (on a dry matter basis), respectively, during the one year study. These concentrations are similar to the present study, though the lowest concentrations documented by Frank et al. (2010a) were lower than the lowest values the present study, probably because they sampled throughout the winter, whereas the present study was conducted only throughout the growing season. The overall WSC means for the present study were 8.18% for perennial ryegrass, 7.49% for tall fescue, 7.14% for KY bluegrass, and 5.60% for orchardgrass (Figure 3.1), which fall well within the range determined by Frank et al. (2010a).

Selim et al. (2015) studied changes in body condition on insulin resistance during the grazing season. They saw that at the end of the grazing period (May to September), horses grazing a cultivated high-yielding pasture compared to a semi-natural grassland had a higher median body condition score, body weight, and larger waist circumference. This is important because obesity is also a risk factor for laminitis (Selim et al., 2015). In their study, the cultivated high-yielding pasture was predominantly tall fescue, timothy (*Phleum pratense* L.), and meadow fescue (*Festuca pratensis* L.), all cool-season grass species similar to those used in the present study. The semi-natural grassland pasture was a meadow/forest mix including meadow foxtail (*Alopecurus pratensis* L.), tufted hairgrass [*Deschampsia cespitosa* (L.) P. Beauv], timothy, white clover (*Trifolium repens* L.), dandelions (*Taraxacum officinale* F.H. Wigg), and meadowsweet [*Filipendula ulmaria* (L.) Maxim], a mix of warm- and cool-season grasses, legumes, and weeds.

The continuation of these types of studies would produce more information on the nonstructural carbohydrate concentrations at which a risk of laminitis increases. Obviously, this is dependent on many factors, including the individual animal and other dietary supplements, but having more information would aid in better animal management. The continuation of this area of research has an important place in the management of grazing animals. Understanding how to properly manage a pasture based on the nutritional demands of animals could save tremendous time and money in the future by preventing illness or disease and maintaining and developing healthy weights.

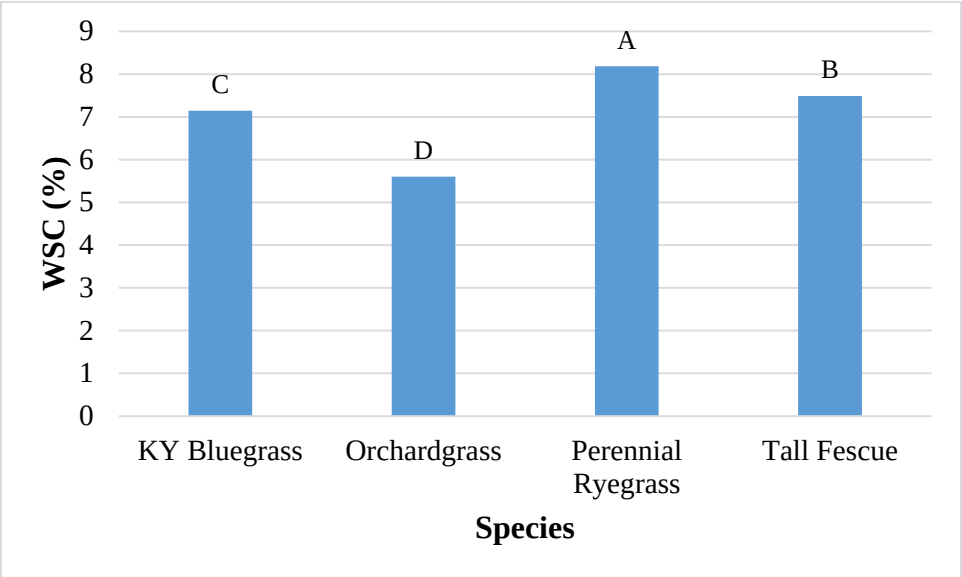


Figure 3.1 Species effect on water-soluble carbohydrates (WSC) of cool-season grasses in central Kentucky from May to November 2015 (n=1531). Means with the same letter are not significantly different at $p<0.05$.

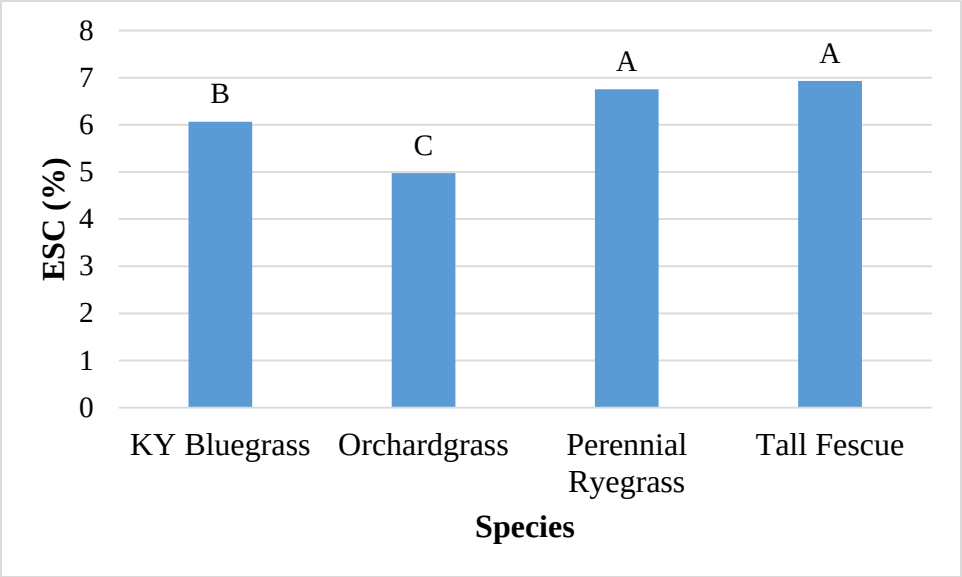


Figure 3.2 Species effect on ethanol-soluble carbohydrates (ESC) of cool-season grasses in central Kentucky from May to November 2015 (n=1531). Means with the same letter are not significantly different at $p<0.05$.

Table 3.5A Cultivar by harvest date by time of day effects on water-soluble carbohydrate concentrations (WSC, %) of cool-season grasses in central Kentucky from 13 May 2015 to 8 July 2015 2015. These results are sorted by harvest date so that each harvest has a separate analysis.

Species	Cultivar	Time of Day	13 May	27 May	10 June	24 June	8 July
KY Bluegrass	Barderby	AM	9.47^{G*}	5.89^{FG}	5.56^{HIJ}	5.00^{H}	3.90 IJK**
		PM	10.86^{F}	5.92^{FG}	7.06^{DE}	6.82^{D}	4.46^{FGH}
	Ginger	AM	11.93^{E}	6.62^{DE}	6.10^{FGH}	5.40^{FGH}	4.77^{EFG}
		PM	13.35^{CD}	6.74^{DE}	7.98^{C}	7.42^{BC}	5.20^{BCDE}
Orchardgrass	Persist	AM	7.63^{K}	3.48^{I}	4.52^{L}	4.04^{I}	3.48^{K}
		PM	8.76^{GHI}	5.35^{H}	6.61^{DEF}	5.66^{EF}	3.89^{IJK}
	Profit	AM	6.72^{L}	3.85^{I}	4.61^{L}	3.84^{I}	3.66^{JK}
		PM	7.83^{JK}	5.17^{H}	6.33^{FG}	5.55^{EFG}	4.31^{GHI}
	Quickdraw	AM	6.08^{L}	3.78^{I}	4.73^{KL}	3.91^{I}	3.55^{JK}
		PM	8.13^{IJK}	5.40^{GH}	6.40^{EFG}	5.73^{EF}	4.04^{HIJ}
Perennial Ryegrass	Aberzest	AM	14.06^{BC}	8.74^{AB}	8.17^{C}	7.02^{CD}	5.48^{BCD}
		PM	15.18^{A}	9.32^{A}	9.73^{A}	8.60^{A}	6.17^{A}
	Calibra	AM	14.44^{AB}	8.10^{C}	7.04^{DE}	5.94^{E}	5.01^{CDE}
		PM	14.96^{AB}	8.23^{BC}	8.65^{BC}	6.98^{CD}	5.58^{B}
	Linn	AM	12.97^{D}	6.34^{EF}	5.20^{JK}	5.71^{EF}	4.96^{DEF}
		PM	13.60^{CD}	6.65^{DE}	7.22^{D}	6.73^{D}	5.41^{BCD}
Tall Fescue	Bronson	AM	9.46^{GH}	5.94^{F}	5.85^{GHI}	5.31^{FGH}	4.35^{GHI}
		PM	11.81^{E}	7.00^{D}	8.90^{B}	7.69^{B}	5.51^{BC}
	Cajun II	AM	8.61^{HIJ}	5.42^{GH}	5.32^{IJ}	5.03^{GH}	4.40^{FGHI}
		PM	10.84^{F}	5.93^{FG}	8.82^{B}	7.17^{BCD}	5.17^{BCDE}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in WSC for each harvest, and red backgrounds designate the group highest in WSC for each harvest.

Table 3.5B Cultivar by harvest date by time of day effects on water-soluble carbohydrates (WSC, %) of cool-season grasses in central Kentucky from 22 July 2015 to 3 November 2015. These results are sorted by harvest date so that each harvest has a separate analysis.

Species	Cultivar	Time of Day	22 July	19 Aug	15 Sept	13 Oct	3 Nov
KY Bluegrass	Barderby	AM	5.19^{HI*}	6.78^{E}	6.07^{EF**}	4.93^{G}	6.13^{J}
		PM	7.44^{E}	8.23^{BCD}	6.72^{DE}	7.86^{DE}	8^{FGH}
	Ginger	AM	6.17^{G}	7.61^{D}	6.43^{EF}	6.25^{F}	9.04^{DE}
		PM	8.14^{D}	9.80^{A}	7.49^{C}	9.93^{B}	10.84^{AB}
Orchardgrass	Persist	AM	4.21^{J}	4.44^{G}	5.82^{F}	4.45^{G}	6.17^{J}
		PM	6.74^{F}	6.16^{EF}	6^{EF}	7.40^{E}	8.49^{EFG}
	Profit	AM	4.08^{J}	4.71^{G}	6.01^{EF}	4.30^{G}	6.66^{IJ}
		PM	6.17^{FG}	6.25^{EF}	6.11^{EF}	8.05^{CDE}	8.74^{EF}
	Quickdraw	AM	4.07^{J}	4.50^{G}	5.86^{F}	4.77^{G}	6.38^{J}
		PM	6.16^{G}	6.12^{F}	6.01^{EF}	7.77^{DE}	8.50^{EFG}
Perennial Ryegrass	Aberzest	AM	6.36^{FG}	8.55^{B}	9.29^{A}	8.43^{CD}	9.86^{CD}
		PM	9.84^{A}	10.07^{A}	9.94^{A}	10.81^{A}	11.65^{A}
	Calibra	AM	5.22^{HI}	5.76^{F}	7.66^{BC}	6.18^{F}	9.32^{CDE}
		PM	8.55^{CD}	8.27^{BC}	8.19^{BC}	10.05^{B}	11.53^{A}
	Linn	AM	5.57^{H}	5.85^{F}	7.45^{CD}	5.87^{F}	7.35^{HI}
		PM	8.05^{D}	7.81^{CD}	7.52^{C}	8.65^{C}	10.10^{BC}
Tall Fescue	Bronson	AM	5.45^{HI}	5.99^{F}	8.33^{B}	6.37^{F}	7.85^{GH}
		PM	9.40^{AB}	8.52^{BC}	9.73^{A}	9.63^{B}	11.01^{A}
	Cajun II	AM	5.13^{I}	5.86^{F}	8.14^{BC}	6.46^{F}	7.91^{FGH}
		PM	9.06^{BC}	8.10^{BCD}	9.45^{A}	9.64^{B}	10.77^{AB}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in WSC for each harvest, and red backgrounds designate the group highest in WSC for each harvest.

Table 3.6 Air temperature, rain, and photosynthetically active radiation (PAR) data for each harvest date recorded by the University of Kentucky North Farm Weather Station.

Date	Time	Average Air Temperature (^oC)*	Total Rain (mm)**	Cumulative Maximum PAR (umol/s/m^2)***
5/13	12am - 8am	10	0	911
	8am - 3pm	15	0	30607
5/27	12am - 8am	20	0	673
	8am - 3pm	24	0	32493
6/10	12am - 8am	17	0	747
	8am - 3pm	27	0	28065
6/24	12am - 8am	20	0	589
	8am - 3pm	24	0	27920
7/8	12am - 8am	21	1	218
	8am - 3pm	23	1	8535
7/22	12am - 8am	19	0	797
	8am - 3pm	23	0	30600
8/19	12am - 8am	23	0	303
	8am - 3pm	25	7	27610
9/15	12am - 8am	12	0	144
	8am - 3pm	21	0	23594
10/13	12am - 8am	15	0	67
	8am - 3pm	16	0	21690
11/3	12am - 8am	9	0	12
	8am - 3pm	16	0	16123

*Average air temperature was calculated by taking the mean of the average air temperature measurements taken every 15 minutes during the time period indicated.
**Total rain was calculated by adding the total rain measurements given every 15 minutes over the course of the time period indicated.
***Cumulative maximum PAR was calculated by adding the maximum PAR of every 15 minutes during the time period indicated.

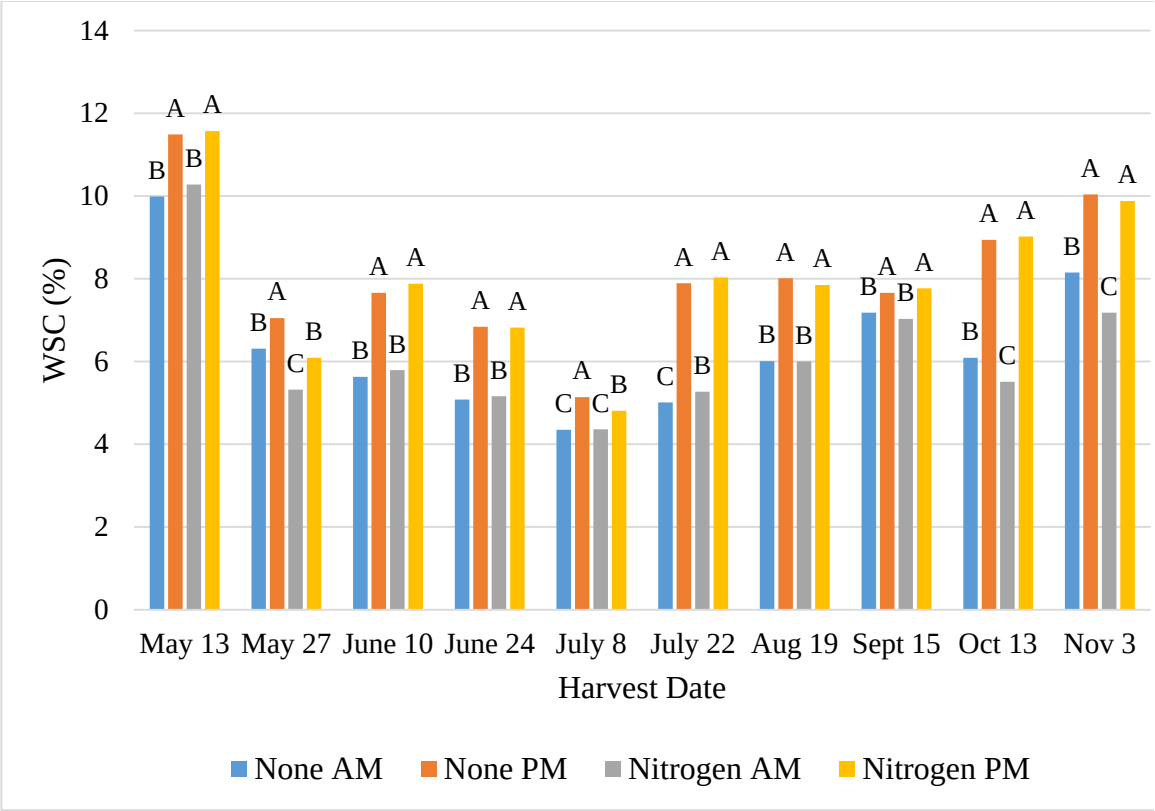


Figure 3.3 Fertility by harvest date by time of day response on water-soluble carbohydrate concentration (WSC, %) of cool-season grasses in central Kentucky from 13 May 2015 to 3 November 2015. These results are sorted by harvest date so that each harvest has a separate analysis. Means with the same letter are not significantly different within a harvest date at $p<0.05$. Nitrogen fertilizer was applied on 16 March (56 kg N ha^{-1}), 13 May (39 kg N ha^{-1}), and 19 August (56 kg N ha^{-1}).

Table 3.7A Cultivar by fertility by harvest date effects on water-soluble carbohydrates (WSC, %) of cool-season grasses in central Kentucky from 13 May 2015 to 8 July 2015. These results are sorted by harvest date so that each harvest has a separate analysis. Nitrogen fertilizer was applied on 16 March (56 kg N ha^{-1}), 13 May (39 kg N ha^{-1}), and 19 August (56 kg N ha^{-1}).

Species	Cultivar	Fertility	13 May	27 May	10 June	24 June	8 July
KY Bluegrass	Barderby	No N	9.89^{EF*}	6.46^{DEF}	6.27^{HIJ}	5.88^{E}	4.22^{EF}
		157 kg N ha-1	10.44^{DE}	5.35^{I}	6.35^{HI}	5.94^{CDE}	4.14^{FG}
	Ginger	No N	12.31^{C}	7.57^{C}	7.10^{DEF}	6.46^{BCD}	5.08^{BCD}
		157 kg N ha-1	12.97^{BC}	5.79^{GHI}	6.98^{EFG}	6.36^{BCDE}	4.90^{CD}
Orchardgrass	Persist	No N	8.44^{G}	4.47^{J**}	5.43^{K}	5.17^{F}	3.80^{FGH}
		157 kg N ha-1	7.96^{GH}	4.36^{J}	5.70^{JK}	4.53^{G}	3.58^{H}
	Profit	No N	6.80^{J}	4.64^{J}	5.27^{K}	4.75^{FG}	4.13^{FGH}
		157 kg N ha-1	7.74^{GHI}	4.37^{J}	5.67^{JK}	4.63^{FG}	3.84^{FGH}
	Quickdraw	No N	7.04^{IJ}	4.77^{J}	5.32^{K}	5.06^{FG}	3.98^{FGH}
		157 kg N ha-1	7.18^{HIJ}	4.41^{J}	5.80^{IJK}	4.59^{G}	3.61^{GH}
Perennial Ryegrass	Aberzest	No N	14.50^{A}	10.34^{A}	9.06^{A}	7.57^{A}	5.99^{A}
		157 kg N ha-1	14.74^{A}	7.72^{C}	8.84^{AB}	8.06^{A}	5.66^{AB}
	Calibra	No N	14.78^{A}	9.61^{B}	8.29^{BC}	6.44^{BCD}	5.29^{BC}
		157 kg N ha-1	14.63^{A}	6.71^{DE}	7.40^{DE}	6.48^{BC}	5.30^{BC}
	Linn	No N	13.38^{B}	6.94^{D}	6.04^{HIJ}	6.00^{CDE}	5.14^{BCD}
		157 kg N ha-1	13.18^{B}	6.06^{FG}	6.38^{GHI}	6.44^{BCD}	5.24^{BCD}
Tall Fescue	Bronson	No N	10.85^{D}	6.63^{DE}	7.06^{EF}	6.35^{BCDE}	5.05^{CD}
		157 kg N ha-1	10.42^{DE}	6.31^{EFG}	7.69^{CD}	6.65^{B}	4.81^{CD}
	Cajun II	No N	9.42^{F}	5.40^{HI}	6.60^{FGH}	5.92^{DE}	4.70^{DE}
		157 kg N ha-1	10.03^{DEF}	5.95^{FGH}	7.54^{DE}	6.28^{BCDE}	4.86^{CD}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in WSC for each harvest, and red backgrounds designate the group highest in WSC for each harvest.

Table 3.7B Cultivar by fertility by harvest date effects on water-soluble carbohydrates (WSC, %) of cool-season grasses in central Kentucky from 22 July 2015 to 3 November 2015. These results are sorted by harvest date so that each harvest has a separate analysis. Nitrogen fertilizer was applied on 16 March (56 kg N ha^{-1}), 13 May (39 kg N ha^{-1}), and 19 August (56 kg N ha^{-1}).

Species	Cultivar	Fertility	22 July	19 Aug	15 Sept	13 Oct	3 Nov
KY Bluegrass	Barderby	No N	5.96^{E*}	7.65^{C}	6.43^{GHI**}	6.02^{H}	6.59^{E}
		157 kg N ha-1	6.66^{D}	7.36^{CDE}	6.37^{GHI}	6.77^{FG}	7.54^{D}
	Ginger	No N	7.17^{BCD}	8.64^{B}	6.80^{FGH}	8.06^{CD}	9.70^{BC}
		157 kg N ha-1	7.13^{BCD}	8.77^{AB}	7.13^{EFG}	8.12^{CD}	10.19^{B}
Orchardgrass	Persist	No N	5.23^{FG}	5.42^{F}	6.06^{HI}	5.94^{H}	7.43^{DE}
		157 kg N ha-1	5.71^{EF}	5.18^{F}	5.75^{I}	5.91^{H}	7.23^{DE}
	Profit	No N	4.96^{G}	5.48^{F}	5.96^{I}	6.43^{GH}	7.73^{D}
		157 kg N ha-1	5.29^{FG}	5.48^{F}	6.16^{HI}	5.92^{H}	7.67^{D}
	Quickdraw	No N	5.20^{FG}	5.26^{F}	6.04^{HI}	6.55^{GH}	7.63^{D}
		157 kg N ha-1	5.03^{G}	5.36^{F}	5.82^{I}	5.99^{H}	7.25^{DE}
Perennial Ryegrass	Aberzest	No N	8.03^{A}	9.35^{A}	10.03^{A}	10.24^{A}	12.15^{A}
		157 kg N ha-1	8.18^{A}	9.27^{AB}	9.20^{AB}	9.01^{B}	9.36^{BC}
	Calibra	No N	6.82^{CD}	7.08^{CDE}	7.62^{DE}	8.70^{BC}	11.70^{A}
		157 kg N ha-1	6.95^{CD}	6.95^{DE}	8.23^{CD}	7.53^{DE}	9.14^{C}
	Linn	No N	6.96^{CD}	6.71^{E}	7.52^{DEF}	7.24^{EF}	9.49^{BC}
		157 kg N ha-1	6.66^{D}	6.95^{DE}	7.46^{DEF}	7.28^{EF}	7.95^{D}
Tall Fescue	Bronson	No N	7.24^{BC}	7.44^{CD}	8.83^{BC}	8.02^{D}	9.52^{BC}
		157 kg N ha-1	7.61^{AB}	7.06^{CDE}	9.23^{AB}	7.98^{D}	9.33^{BC}
	Cajun II	No N	6.91^{CD}	7.06^{CDE}	8.93^{BC}	7.96^{D}	9.04^{C}
		157 kg N ha-1	7.28^{BC}	6.90^{DE}	8.66^{BC}	8.14^{CD}	9.64^{BC}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in WSC for each harvest, and red backgrounds designate the group highest in WSC for each harvest.

Table 3.8A Cultivar by harvest date by time of day effects on ethanol-soluble carbohydrates (ESC, %) of cool-season grasses in central Kentucky from 13 May 2015 to 8 July 2015. These results are sorted by harvest date so that each harvest has a separate analysis.

Species	Cultivar	Time of Day	13 May	27 May	10 June	24 June	8 July
KY Bluegrass	Barderby	AM	5.89^{IJ*}	3.77^{G}	4.82^{IJK}	4.14^{G}	3.60^{CDE}
		PM	8.52^{CD}	5.60^{B}	7.01^{CD}	7.17^{BC}	4.69^{B}
	Ginger	AM	5.57^{JK}	3.17^{HI}	4.37^{KL}	4.20^{G}	3.69^{CDE}
		PM	8.39^{CD}	5.20^{BCD}	6.74^{DE}	7.42^{B}	4.48^{B}
Orchardgrass	Persist	AM	5.22^{K}	2.64^{JK**}	3.76^{MN}	2.97^{H}	2.98^{FG}
		PM	7.07^{FG}	4.76^{CDE}	6.17^{EFG}	5.40^{D}	3.67^{CDE}
	Profit	AM	4.27^{L}	2.27^{K}	3.65^{N}	3.05^{H}	2.77^{G}
		PM	5.79^{JK}	4.57^{DE}	5.83^{GH}	5.27^{DE}	3.81^{CD}
	Quickdraw	AM	3.80^{L}	2.82^{IJ}	3.85^{MN}	3.03^{H}	2.80^{G}
		PM	6.52^{GHI}	4.93^{BCDE}	5.92^{FG}	5.43^{D}	3.65^{CDE}
Perennial Ryegrass	Aberzest	AM	7.48^{EF}	5.11^{BCD}	5.19^{HI}	4.72^{EF}	3.47^{DE}
		PM	10.66^{AB}	7.42^{A}	8.14^{AB}	7.70^{AB}	5.31^{A}
	Calibra	AM	8.15^{DE}	5.13^{BCD}	5.14^{I}	4.32^{FG}	3.38^{EF}
		PM	10.74^{A}	7.28^{A}	7.75^{BC}	7.20^{BC}	4.71^{B}
	Linn	AM	8.70^{CD}	3.92^{FG}	4.18^{LM}	4.06^{G}	3.32^{EF}
		PM	10.45^{AB}	5.58^{B}	6.65^{DEF}	6.59^{C}	4.79^{B}
Tall Fescue	Bronson	AM	6.68^{FGH}	4.35^{EF}	5.09^{IJ}	4.47^{FG}	3.92^{C}
		PM	10.02^{B}	6.68^{A}	8.80^{A}	8.45^{A}	4.67^{B}
	Cajun II	AM	6.08^{HIJ}	3.56^{GH}	4.57^{JKL}	4.25^{FG}	3.65^{CDE}
		PM	8.93^{C}	5.40^{BC}	8.68^{A}	8.17^{A}	4.58^{B}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in ESC for each harvest, and red backgrounds designate the group highest in ESC for each harvest.

Table 3.8B Cultivar by harvest date by time of day effects on ethanol-soluble carbohydrates (ESC, %) of cool-season grasses in central Kentucky from 22 July 2015 to 3 November 2015. These results are sorted by harvest date so that each harvest has a separate analysis.

Species	Cultivar	Time of Day	22 July	19 Aug	15 Sept	13 Oct	3 Nov
KY Bluegrass	Barderby	AM	5.60^{GH*}	5.69^{DE}	5.22^{EFG}	3.90^{HI**}	5.55^{K}
		PM	8.64^{C}	8.12^{AB}	5.96^{CDE}	8.19^{D}	8.88^{FG}
	Ginger	AM	5.41^{H}	4.84^{FG}	5.09^{FGH}	4.19^{GH}	6.86^{IJ}
		PM	8.26^{C}	7.69^{BC}	6.08^{CD}	9.47^{C}	10.43^{CD}
Orchardgrass	Persist	AM	4.09^{I}	3.64^{J}	4.41^{HI}	4.18^{GH}	5.54^{K}
		PM	7.03^{D}	5.55^{DE}	4.67^{GHI}	7.87^{D}	9.97^{DE}
	Profit	AM	3.79^{I}	3.76^{J}	4.21^{I}	3.59^{I}	5.36^{K}
		PM	6.29^{EF}	5.93^{D}	4.69^{GHI}	8.23^{D}	9.29^{EF}
	Quickdraw	AM	4.11^{I}	3.91^{IJ}	4.63^{GHI}	4.29^{GH}	5.34^{K}
		PM	6.66^{DE}	5.88^{D}	4.96^{FGHI}	8.11^{D}	9.34^{EF}
Perennial Ryegrass	Aberzest	AM	5.82^{FG}	5.29^{EF}	6.07^{CD}	5.72^{E}	7.59^{HI}
		PM	10.19^{A}	8.64^{A}	7.13^{B}	10.52^{AB}	11.21^{BC}
	Calibra	AM	5.28^{H}	4.35^{HI}	5.51^{DEF}	5.47^{EF}	8.27^{GH}
		PM	9.25^{B}	7.96^{B}	6.70^{BC}	10.91^{A}	12.38^{A}
	Linn	AM	5.27^{H}	3.87^{J}	4.89^{FGHI}	4.62^{G}	6.53^{J}
		PM	8.66^{C}	7.33^{C}	5.61^{DEF}	9.84^{BC}	11.18^{BC}
Tall Fescue	Bronson	AM	5.89^{FG}	4.72^{GH}	6.98^{B}	5.28^{EF}	7.61^{H}
		PM	10.66^{A}	8.07^{AB}	8.36^{A}	9.74^{C}	11.82^{AB}
	Cajun II	AM	5.60^{GH}	4.55^{GH}	6.48^{BC}	5.16^{F}	7.70^{H}
		PM	10.12^{A}	7.72^{BC}	8.05^{A}	9.33^{C}	11.63^{AB}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in ESC for each harvest, and red backgrounds designate the group highest in ESC for each harvest.

Table 3.9A Cultivar by fertility treatment by harvest date effects on ethanol-soluble carbohydrates (ESC, %) of cool-season grasses in central Kentucky from 13 May 2015 to 8 July 2015. These results are sorted by harvest date so that each harvest has a separate analysis. Nitrogen fertilizer was applied on 16 March (56 kg N ha^{-1}), 13 May (39 kg N ha^{-1}), and 19 August (56 kg N ha^{-1}).

Species	Cultivar	Fertility	13 May	27 May	10 June	24 June	8 July
KY Bluegrass	Barderby	No N	6.97^{EF*}	4.76^{BC}	5.78^{EFG}	5.75^{CDEF}	4.15^{ABC**}
		157 kg N ha-1	7.44^{CDE}	4.61^{BCD}	6.05^{DE}	5.56^{DEF}	4.14^{ABC}
	Ginger	No N	6.73^{FG}	4.15^{CDE}	5.21^{FGHI}	5.71^{CDEF}	3.95^{C}
		157 kg N ha-1	7.23^{DEF}	4.21^{CDE}	5.90^{DEF}	5.91^{ABCDE}	4.21 ^{ABC}
Orchardgrass	Persist	No N	6.28^{GH}	3.16^{G}	4.87^{HIJ}	4.49^{G}	3.21^{D}
		157 kg N ha-1	6.02^{HI}	4.24^{CDE}	5.06^{HI}	3.87^{H}	3.44^{D}
	Profit	No N	4.58^{K}	2.75^{G}	4.36^{J}	4.21^{GH}	3.12^{D}
		157 kg N ha-1	5.48^{IJ}	4.09^{DE}	5.13^{GHI}	4.11^{GH}	3.47^{D}
	Quickdraw	No N	5.05^{JK}	3.23^{FG}	4.53^{IJ}	4.39^{GH}	3.15^{D}
		157 kg N ha-1	5.27^{J}	4.52^{BCD}	5.23^{FGH}	4.07^{GH}	3.30^{D}
Perennial Ryegrass	Aberzest	No N	8.76^{B}	6.08^{A}	6.25^{DE}	6.24^{ABC}	4.28^{ABC}
		157 kg N ha-1	9.38^{AB}	6.45^{A}	7.08^{ABC}	6.18^{ABCD}	4.50^{A}
	Calibra	No N	9.27^{AB}	6.19^{A}	6.39^{CDE}	5.68^{CDEF}	4.04^{BC}
		157 kg N ha-1	9.63^{A}	6.22^{A}	6.50^{BCD}	5.84^{BCDEF}	4.05^{BC}
	Linn	No N	9.62^{A}	4.36^{CDE}	4.99^{HIJ}	5.43^{EF}	4.04^{BC}
		157 kg N ha-1	9.54^{A}	5.15^{B}	5.84^{DEF}	5.22^{F}	4.07^{ABC}
Tall Fescue	Bronson	No N	8.78^{B}	5.17^{B}	6.50^{BCD}	6.43^{AB}	4.20^{ABC}
		157 kg N ha-1	7.93^{C}	5.87^{A}	7.39^{A}	6.50^{A}	4.40^{AB}
	Cajun II	No N	7.30^{CDEF}	3.84^{EF}	6.12^{DE}	6.19^{ABC}	4.07^{ABC}
		157 kg N ha-1	7.72^{CD}	5.12^{B}	7.13^{AB}	6.24^{ABC}	4.16^{ABC}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in ESC for each harvest, and red backgrounds designate the group highest in ESC for each harvest.

Table 3.9B Cultivar by fertility treatment by harvest date effects on ethanol-soluble carbohydrates (ESC, %) of cool-season grasses in central Kentucky from 22 July 2015 to 3 November 2015. These results are sorted by harvest date so that each harvest has a separate analysis. Nitrogen fertilizer was applied on 16 March (56 kg N ha^{-1}), 13 May (39 kg N ha^{-1}), and 19 August (56 kg N ha^{-1}).

Species	Cultivar	Fertility	22 July	19 Aug	15 Sept	13 Oct	3 Nov
KY Bluegrass	Barderby	No N	7.02^{EFG*}	6.95^{AB**}	5.90^{CD}	5.76^{IJ}	6.70^{IJ}
		157 kg N ha-1	7.21^{DEF}	6.86^{ABC}	5.28^{DEFG}	6.33^{GHI}	7.73^{GH}
	Ginger	No N	6.90^{FG}	6.18^{DE}	5.35^{DEF}	6.42^{FGH}	7.77^{GH}
		157 kg N ha-1	6.78^{FG}	6.35^{CD}	5.82^{CD}	7.24^{CDE}	9.53^{BCDE}
Orchardgrass	Persist	No N	5.21^{IJ}	4.67^{H}	4.53^{GH}	5.14^{JK}	7.33^{HI}
		157 kg N ha-1	5.91^{H}	4.52^{H}	4.55^{FGH}	6.92^{DEFG}	8.18^{FG}
	Profit	No N	4.86^{J}	4.69^{H}	4.36^{H}	5.12^{K}	6.21^{J}
		157 kg N ha-1	5.21^{IJ}	5.00^{GH}	4.53^{GH}	6.71^{EFGH}	8.44^{FG}
	Quickdraw	No N	5.40^{I}	4.76^{H}	4.91^{EFGH}	5.38^{JK}	6.80^{IJ}
		157 kg N ha-1	5.38^{I}	5.02^{GH}	4.68^{FGH}	7.02^{DEF}	7.88^{GH}
Perennial Ryegrass	Aberzest	No N	7.88^{BC}	6.79^{ABC}	7.14^{AB}	7.49^{CD}	8.96^{EF}
		157 kg N ha-1	8.14^{AB}	7.13^{A}	6.06^{CD}	8.75^{A}	9.85^{ABCD}
	Calibra	No N	7.04^{EFG}	5.90^{DEF}	5.81^{CD}	8.23^{AB}	10.38^{A}
		157 kg N ha-1	7.48^{CDE}	6.41^{BCD}	6.39^{BC}	8.14^{AB}	10.27^{AB}
	Linn	No N	7.23^{DEF}	5.49^{FG}	4.96^{EFGH}	6.76^{EFG}	8.74^{EF}
		157 kg N ha-1	6.70^{G}	5.71^{EF}	5.53^{DE}	7.70^{BC}	8.97^{DEF}
Tall Fescue	Bronson	No N	7.93^{BC}	6.42^{BCD}	7.58^{A}	6.53^{FGH}	9.43^{CDE}
		157 kg N ha-1	8.61^{A}	6.37^{CD}	7.75^{A}	8.49^{A}	10.00^{ABC}
	Cajun II	No N	7.68^{BCD}	6.24^{DE}	7.17^{AB}	6.11^{HI}	8.87^{EF}
		157 kg N ha-1	8.03^{B}	6.04^{DE}	7.35^{A}	8.37^{A}	10.47^{A}

*Means with the same letter are not significantly different within a harvest date at $p<0.05$.
**Yellow backgrounds designate the group lowest in ESC for each harvest, and red backgrounds designate the group highest in ESC for each harvest.

Appendix A. Detailed laboratory methods for creating the phenol reagent for the phenol-sulfuric acid assays used to quantify water-soluble and ethanol-soluble carbohydrates.

Five percent (w/w) phenol reagent was created in the fume hood by melting phenol at 40-44^{o}C in a water bath on a hot plate. The amount needed for desired volume was calculated. For example, for 500 mL, 25 g phenol is needed based on a phenol density of 1.07 g/mL. For this amount, 23.4 mL phenol was measured into a 25 mL graduated cylinder, then poured into a 1 L Pyrex bottle. 477 mL (~476 mL) water was measured into a 1 L graduated cylinder. This water was used to rinse the 25 mL cylinder into the Pyrex bottle (multiple 25 mL aliquots). The bottle was swirled to mix the contents, and stored at 4^{o}C in foil. This reagent can be stable for three years.

Appendix B. Kentucky bluegrass wet chemistry data of water-soluble and ethanol-soluble carbohydrate concentrations used for near infrared reflectance spectroscopy equation calibration, ordered by cultivar.

Sample ID	Cultivar	Plot	Harvest Date	Time of Day	Fertility	Block	% Mean WSC	% Mean ESC
509032	Barderby	509	5/13	PM	None	3	11.11	8.04
609042	Barderby	609	5/27	PM	N	3	5.40	4.87
401042	Barderby	401	5/27	PM	None	2	7.63	6.81
609052	Barderby	609	6/10	PM	N	3	7.89	6.69
704052	Barderby	704	6/10	PM	N	4	8.81	7.60
107062	Barderby	107	6/24	PM	None	1	4.39	7.38
207071	Barderby	207	7/8	AM	N	1	3.17	4.20
609071	Barderby	609	7/8	AM	N	3	3.79	2.69
609081	Barderby	609	7/22	AM	N	3	4.91	6.15
609082	Barderby	609	7/22	PM	N	3	7.97	9.21
401081	Barderby	401	7/22	AM	None	2	5.31	5.65
401082	Barderby	401	7/22	PM	None	2	7.41	7.71
509082	Barderby	509	7/22	PM	None	3	6.82	8.16
609092	Barderby	609	8/19	PM	N	3	7.63	8.10
804091	Barderby	804	8/19	AM	None	4	5.75	5.50
609101	Barderby	609	9/15	AM	N	3	5.75	3.89
207102	Barderby	207	9/15	PM	N	1	6.41	4.63
107101	Barderby	107	9/15	AM	None	1	7.05	6.21
804101	Barderby	804	9/15	AM	None	4	7.49	5.50
107102	Barderby	107	9/15	PM	None	1	7.71	5.35
509102	Barderby	509	9/15	PM	None	3	6.25	4.20
301111	Barderby	301	10/13	AM	N	2	3.85	4.13
509111	Barderby	509	10/13	AM	None	3	3.46	2.85
804111	Barderby	804	10/13	AM	None	4	5.20	4.19
207121	Barderby	207	11/3	AM	N	1	5.89	5.90
207122	Barderby	207	11/3	PM	N	1	10.17	10.02
509121	Barderby	509	11/3	AM	None	3	4.22	3.80
107122	Barderby	107	11/3	PM	None	1	5.24	6.37
206031	Ginger	206	5/13	AM	N	1	9.29	6.35
303032	Ginger	303	5/13	PM	N	2	13.13	8.37
403032	Ginger	403	5/13	PM	None	2	13.71	8.01
706041	Ginger	706	5/27	AM	N	4	5.54	3.81
806042	Ginger	806	5/27	PM	None	4	7.84	5.55
106051	Ginger	106	6/10	AM	None	1	6.70	4.77
106052	Ginger	106	6/10	PM	None	1	10.36	5.56
706061	Ginger	706	6/24	AM	N	4	6.24	4.99
706062	Ginger	706	6/24	PM	N	4	6.82	8.85
403061	Ginger	403	6/24	AM	None	2	5.27	4.37

403062	Ginger	403	6/24	PM	None	2	4.95	7.27
106072	Ginger	106	7/8	PM	None	1	4.57	3.34
806081	Ginger	806	7/22	AM	None	4	5.58	5.28
106082	Ginger	106	7/22	PM	None	1	7.40	8.93
806082	Ginger	806	7/22	PM	None	4	9.60	11.36
303091	Ginger	303	8/19	AM	N	2	6.22	5.84
806091	Ginger	806	8/19	AM	None	4	6.78	4.67
106101	Ginger	106	9/15	AM	None	1	7.87	5.05
106102	Ginger	106	9/15	PM	None	1	7.86	5.16
403111	Ginger	403	10/13	AM	None	2	5.46	4.29
508112	Ginger	508	10/13	PM	None	3	10.02	10.91
508121	Ginger	508	11/3	AM	None	3	11.47	5.72
806121	Ginger	806	11/3	AM	None	4	7.35	5.39

Appendix C. Orchardgrass wet chemistry data of water-soluble and ethanol-soluble carbohydrate concentrations used for near infrared reflectance spectroscopy equation calibration, ordered by cultivar.

Sample ID	Cultivar	Plot	Harvest Date	Time of Day	Fertility	Block	% Mean WSC	% Mean ESC
204011	Persist	204	4/15	AM	N	1	4.57	5.27
809011	Persist	809	4/15	AM	None	4	3.74	3.31
104012	Persist	104	4/15	PM	None	1	6.40	4.77
709061	Persist	709	6/24	AM	N	4	2.43	3.41
104062	Persist	104	6/24	PM	None	1	5.55	4.98
402071	Persist	402	7/8	AM	None	2	3.27	1.83
809081	Persist	809	7/22	AM	None	4	4.34	4.41
709092	Persist	709	8/19	PM	N	4	5.88	6.20
505091	Persist	505	8/19	AM	None	3	4.75	4.07
505092	Persist	505	8/19	PM	None	3	5.51	5.19
809092	Persist	809	8/19	PM	None	4	5.83	5.79
204101	Persist	204	9/15	AM	N	1	6.29	4.40
605102	Persist	605	9/15	PM	N	3	7.14	5.70
204112	Persist	204	10/13	PM	N	1	7.70	8.93
402112	Persist	402	10/13	PM	None	2	6.64	6.54
809121	Persist	809	11/3	AM	None	4	4.90	4.70
809122	Persist	809	11/3	PM	None	4	10.82	10.31
305012	Profit	305	4/15	PM	N	2	6.53	6.67
305031	Profit	305	5/13	AM	N	2	8.41	5.16
707032	Profit	707	5/13	PM	N	4	8.17	5.74
103042	Profit	103	5/27	PM	None	1	5.15	4.29
807042	Profit	807	5/27	PM	None	4	5.67	3.53
203051	Profit	203	6/10	AM	N	1	6.60	4.43
510051	Profit	510	6/10	AM	None	3	4.65	3.68
103061	Profit	103	6/24	AM	None	1	5.52	3.40
510061	Profit	510	6/24	AM	None	3	4.49	.
203071	Profit	203	7/8	AM	N	1	3.37	2.24
203072	Profit	203	7/8	PM	N	1	3.94	3.07
510081	Profit	510	7/22	AM	None	3	4.48	4.26
405082	Profit	405	7/22	PM	None	2	5.42	7.11
610092	Profit	610	8/19	PM	N	3	5.56	5.61
405091	Profit	405	8/19	AM	None	2	4.05	3.16
405092	Profit	405	8/19	PM	None	2	5.59	5.77
510101	Profit	510	9/15	AM	None	3	6.76	4.72
203111	Profit	203	10/13	AM	N	1	3.97	4.59
405112	Profit	405	10/13	PM	None	2	10.53	8.10
510121	Profit	510	11/3	AM	None	3	7.06	5.06

310012	Quickdraw	310	4/15	PM	N	2	6.75	6.11
105011	Quickdraw	105	4/15	AM	None	1	4.24	5.26
501011	Quickdraw	501	4/15	AM	None	3	4.60	4.94
410012	Quickdraw	410	4/15	PM	None	2	7.09	7.22
501012	Quickdraw	501	4/15	PM	None	3	6.08	6.04
601032	Quickdraw	601	5/13	PM	N	3	7.31	7.76
710032	Quickdraw	710	5/13	PM	N	4	7.93	6.37
601042	Quickdraw	601	5/27	PM	N	3	6.42	4.73
410041	Quickdraw	410	5/27	AM	None	2	4.48	2.61
105042	Quickdraw	105	5/27	PM	None	1	5.98	3.58
710052	Quickdraw	710	6/10	PM	N	4	7.70	6.18
310061	Quickdraw	310	6/24	AM	N	2	4.29	3.40
310071	Quickdraw	310	7/8	AM	N	2	4.31	1.95
205081	Quickdraw	205	7/22	AM	N	1	4.79	4.41
601081	Quickdraw	601	7/22	AM	N	3	4.26	4.63
205082	Quickdraw	205	7/22	PM	N	1	6.09	7.76
810082	Quickdraw	810	7/22	PM	None	4	5.79	7.34
205101	Quickdraw	205	9/15	AM	N	1	7.23	5.36
810102	Quickdraw	810	9/15	PM	None	4	6.49	5.37
410111	Quickdraw	410	10/13	AM	None	2	4.63	3.91
205122	Quickdraw	205	11/3	PM	N	1	8.55	9.34
410122	Quickdraw	410	11/3	PM	None	2	8.24	7.21
501122	Quickdraw	501	11/3	PM	None	3	9.05	9.65

Appendix D. Perennial ryegrass wet chemistry data of water-soluble and ethanol-soluble carbohydrate concentrations used for near infrared reflectance spectroscopy equation calibration, ordered by cultivar.

Sample ID	Cultivar	Plot	Harvest Date	Time of Day	Fertility	Block	% Mean WSC	% Mean ESC
603012	Aberzest	603	4/15	PM	N	3	7.94	8.09
503011	Aberzest	503	4/15	AM	None	3	7.44	6.84
808012	Aberzest	808	4/15	PM	None	4	9.13	8.47
308031	Aberzest	308	5/13	AM	N	2	13.67	6.78
603032	Aberzest	603	5/13	PM	N	3	16.61	8.50
708032	Aberzest	708	5/13	PM	N	4	14.57	10.69
110042	Aberzest	110	5/27	PM	None	1	9.89	5.95
308052	Aberzest	308	6/10	PM	N	2	10.20	8.12
408052	Aberzest	408	6/10	PM	None	2	10.06	7.30
708061	Aberzest	708	6/24	AM	N	4	4.76	4.87
808061	Aberzest	808	6/24	AM	None	4	3.79	5.52
110072	Aberzest	110	7/8	PM	None	1	7.24	5.10
408081	Aberzest	408	7/22	AM	None	2	6.45	4.70
708091	Aberzest	708	8/19	AM	N	4	7.09	5.25
408092	Aberzest	408	8/19	PM	None	2	9.24	9.14
308102	Aberzest	308	9/15	PM	N	2	9.12	6.38
503101	Aberzest	503	9/15	AM	None	3	11.62	6.57
708121	Aberzest	708	11/3	AM	N	4	8.52	8.74
408122	Aberzest	408	11/3	PM	None	2	11.80	9.89
304011	Calibra	304	4/15	AM	N	2	4.23	4.25
606011	Calibra	606	4/15	AM	N	3	5.53	6.37
702011	Calibra	702	4/15	AM	N	4	6.33	6.42
404012	Calibra	404	4/15	PM	None	2	8.91	8.47
606032	Calibra	606	5/13	PM	N	3	15.25	10.90
404031	Calibra	404	5/13	AM	None	2	13.59	7.61
209041	Calibra	209	5/27	AM	N	1	8.23	4.67
506052	Calibra	506	6/10	PM	None	3	8.20	6.70
304062	Calibra	304	6/24	PM	N	2	5.17	8.00
506061	Calibra	506	6/24	AM	None	3	6.28	4.26
404062	Calibra	404	6/24	PM	None	2	5.47	7.01
209071	Calibra	209	7/8	AM	N	1	3.79	2.56
109072	Calibra	109	7/8	PM	None	1	5.69	3.57
109082	Calibra	109	7/22	PM	None	1	7.92	10.30
802082	Calibra	802	7/22	PM	None	4	8.24	9.62
209091	Calibra	209	8/19	AM	N	1	6.86	4.96
304091	Calibra	304	8/19	AM	N	2	6.27	4.80
802101	Calibra	802	9/15	AM	None	4	7.74	4.63

209111	Calibra	209	10/13	AM	N	1	6.57	6.04
506112	Calibra	506	10/13	PM	None	3	12.24	12.14
108011	Linn	108	4/15	AM	None	1	6.58	6.49
306031	Linn	306	5/13	AM	N	2	13.57	8.42
108032	Linn	108	5/13	PM	None	1	16.12	8.83
108041	Linn	108	5/27	AM	None	1	8.57	4.30
108042	Linn	108	5/27	PM	None	1	7.12	5.20
108051	Linn	108	6/10	AM	None	1	6.07	3.31
602061	Linn	602	6/24	AM	N	3	5.35	4.08
208062	Linn	208	6/24	PM	N	1	5.02	6.72
108061	Linn	108	6/24	AM	None	1	5.27	2.98
502061	Linn	502	6/24	AM	None	3	5.48	4.83
306071	Linn	306	7/8	AM	N	2	4.90	3.58
502071	Linn	502	7/8	AM	None	3	3.96	2.70
406072	Linn	406	7/8	PM	None	2	5.40	3.63
502072	Linn	502	7/8	PM	None	3	5.60	3.22
306082	Linn	306	7/22	PM	N	2	7.70	7.46
108081	Linn	108	7/22	AM	None	1	5.85	5.33
602091	Linn	602	8/19	AM	N	3	6.04	4.49
108092	Linn	108	8/19	PM	None	1	7.52	9.13
801092	Linn	801	8/19	PM	None	4	7.95	8.07
306101	Linn	306	9/15	AM	N	2	7.89	5.17
406101	Linn	406	9/15	AM	None	2	7.11	4.37
801102	Linn	801	9/15	PM	None	4	9.00	5.61
208112	Linn	208	10/13	PM	N	1	11.28	10.22
406111	Linn	406	10/13	AM	None	2	5.97	4.85
108121	Linn	108	11/3	AM	None	1	7.51	6.85
406121	Linn	406	11/3	AM	None	2	7.90	7.16

Appendix E. Tall fescue wet chemistry data of water-soluble and ethanol-soluble carbohydrate concentrations used for near infrared reflectance spectroscopy equation calibration, ordered by cultivar.

Sample ID	Cultivar	Plot	Harvest Date	Time of Day	Fertility	Block	% Mean WSC	% Mean ESC
201011	Bronson	201	4/15	AM	N	1	5.06	5.69
607011	Bronson	607	4/15	AM	N	3	4.05	4.48
703012	Bronson	703	4/15	PM	N	4	6.69	6.50
201032	Bronson	201	5/13	PM	N	1	11.62	8.88
607032	Bronson	607	5/13	PM	N	3	12.60	11.32
201041	Bronson	201	5/27	AM	N	1	4.87	3.41
409051	Bronson	409	6/10	AM	None	2	6.76	5.73
507051	Bronson	507	6/10	AM	None	3	5.83	4.58
803051	Bronson	803	6/10	AM	None	4	6.91	5.16
201061	Bronson	201	6/24	AM	N	1	4.80	3.75
309061	Bronson	309	6/24	AM	N	2	4.73	4.17
703071	Bronson	703	7/8	AM	N	4	3.42	2.71
101071	Bronson	101	7/8	AM	None	1	3.32	2.75
803071	Bronson	803	7/8	AM	None	4	3.60	3.60
309082	Bronson	309	7/22	PM	N	2	8.90	11.07
703092	Bronson	703	8/19	PM	N	4	7.73	7.94
607101	Bronson	607	9/15	AM	N	3	7.65	5.84
309112	Bronson	309	10/13	PM	N	2	9.98	10.30
803111	Bronson	803	10/13	AM	None	4	5.56	4.84
101121	Bronson	101	11/3	AM	None	1	7.44	7.70
803121	Bronson	803	11/3	AM	None	4	8.34	8.26
407012	Cajun II	407	4/15	PM	None	2	8.55	7.97
307031	Cajun II	307	5/13	AM	N	2	8.22	6.55
604032	Cajun II	604	5/13	PM	N	3	12.36	9.07
805032	Cajun II	805	5/13	PM	None	4	11.11	7.82
705042	Cajun II	705	5/27	PM	N	4	6.58	6.47
504041	Cajun II	504	5/27	AM	None	3	5.67	3.46
202051	Cajun II	202	6/10	AM	N	1	5.49	5.10
202052	Cajun II	202	6/10	PM	N	1	10.45	8.87
705062	Cajun II	705	6/24	PM	N	4	6.87	10.32
102061	Cajun II	102	6/24	AM	None	1	4.62	3.72
504062	Cajun II	504	6/24	PM	None	3	5.70	7.52
202072	Cajun II	202	7/8	PM	N	1	4.80	3.40
102082	Cajun II	102	7/22	PM	None	1	8.65	10.32
407091	Cajun II	407	8/19	AM	None	2	5.16	5.01
407092	Cajun II	407	8/19	PM	None	2	7.58	8.91
202102	Cajun II	202	9/15	PM	N	1	9.02	7.89

407101	Cajun II	407	9/15	AM	None	2	9.02	6.33
102112	Cajun II	102	10/13	PM	None	1	8.48	8.32
805112	Cajun II	805	10/13	PM	None	4	10.39	8.28
307121	Cajun II	307	11/3	AM	N	2	10.18	9.13
202122	Cajun II	202	11/3	PM	N	1	11.61	12.18
102122	Cajun II	102	11/3	PM	None	1	8.78	10.28
805122	Cajun II	805	11/3	PM	None	4	9.00	10.45

References

Ball, D. M., M. Collins, G. D. Lacefield, N. P. Martin, D. A. Mertens, K. E. Olson, D. H. Putnam, D. J. Undersander, and M. W. Wolf. 2001. Understanding forage quality. American Farm Bureau Federation Publication, Park Ridge, IL.

Belknap, J. K. 2017. Laminitis: an overview. In: Equine laminitis. John Wiley & Sons inc., p. 11-12.

Borer, K. E., S. R. Bailey, N. J. Menzies-Gow, P. A. Harris, and J. Elliott. 2012. Effect of feeding glucose, fructose, and inulin on blood glucose and insulin concentrations in normal ponies and those predisposed to laminitis. Journal of Animal Science. 90:3003-3011.

Brown, R. H., and R. E. Blaser. 1965. Relationships between reserve carbohydrate accumulation and growth rate in orchardgrass and tall fescue. Crop Science. 5:577-582.

Brushwood, D. E. 2000. Modification of the potassium ferricyanide reducing sugar test for sugars from extracts of cotton fiber. Journal of Cotton Science. 4:202-209.

Chatterton, N. J., and P. A. Harrison. 1997. Fructan oligomers in *Poa ampla*. New Phytologist. 136:3-10.

Chatterton, N. J, P. A. Harrison, J. H. Bennett, and K. H. Asay. 1989. Carbohydrate partitioning in 185 accessions of Gramineae grown under warm and cool temperatures. Plant Physiology. 134:169-179.

Ciavarella, T. A., R. J. Simpson, H. Dove, B. J. Leury, and I. M. Sims. 2000. Diurnal changes in the concentration of water-soluble carbohydrates in *Phalaris aquatica* L. pasture in spring, and the effect of short-term shading. Australian Journal of Agricultural Research. 51:749-756.

Ciurczak, E. W. 2002. Growth of near-infrared spectroscopy in pharmaceutical and medical sciences. In: Biomedical Nanotechnology Architectures and Applications Proceedings, San Jose, CA.

Cubitt, T. A., W. B. Staniar, D. S. Kronfield, B. M. Byrd, and P. A. Harris. 2007. Environmental effects on nutritive value of equine pastures in northern virginia. Pferdeheilkunde. 23:151-154.

Dambergs, B., M. Esler, and M. Gishen. 2004. Analysis of beverages and brewing products. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 465-485.

Delwiche, S. R. 2004. Analysis of small grain crops. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 269-320.

Downey, G., and K. I. Hildrum. 2004. Analysis of meats. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 599-632.

DuBois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry. 28:350-356.

Dyer, D. J. 2004. Analysis of oilseeds and coarse grains. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 321-344.

Eades, S. C. 2010. Overview of current laminitis research. Veterinary Clinics of North America: Equine Practice. 26:51-63.

Engle, D. M., and C. D. Bonham. 1980. Nonstructural carbohydrates in roots of gambel oak sprouts following herbicide treatment. Journal of Range Management. 33:390-394.

Fisher D. S., H. F. Maryland, and J. C. Burns. 1999. Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. Journal of Animal Science. 77:762-768.

Frank, N., S. B. Elliot, K. A. Chameroy, F. Tóth, N. S. Chumbler, and R. McClamroch. 2010a. Association of season and pasture grazing with blood hormone and metabolite concentrations in horses with presumed pituitary pars intermedia dysfunction. Journal of Veterinary Internal Medicine. 24:1167-1175.

Frank, N., R. J. Geor, S. R. Bailey, A. E. Durham, and P. J. Johnson. 2010b. Equine metabolic syndrome. Journal of Veterinary Internal Medicine. 24:467-475.

Frossard, R., F. X. Stadelmann, and J. Niederhauser. 1989. Effects of different heavy metals on fructan, sugar and starch content of ryegrass. Journal of Plant Physiology. 134:180-185.

Garner, H. E., D. P. Hutcheson, J. R. Coffman, A. W. Hahn, and C. Salem. 1977. Lactic acidosis: a factor associated with equine laminitis. Journal of Animal Science. 45:1037-1041.

Garrido-Varo, A., J. García-Olmo, and M. D. Pérez-Marin. 2004. Applications in fats and oils. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 487-558.

Geor, R. J. 2010. Current concepts on the pathophysiology of pasture-associated laminitis. Veterinary Clinics of North America: Equine Practice. 26:265-276.

Geor, R., J. deSilva, K. Meyers, T. Smith, and P. Harris. 2010. Effects of short-term adaptation to dietary carbohydrates on glucose and insulin dynamics in healthy and overweight/obese, insulin resistant mares. Journal of Equine Veterinary Science. 30:97.

Giangiacomo, R., and T. M.P. Cattaneo. 2004. Analysis of dairy and eggs. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 559-597.

Hall, M. B. 2014. Selection of an empirical detection method for determination of water-soluble carbohydrates in feedstuffs for application in ruminant nutrition. Animal Feed Science and Technology. 198:28-37.

Hartwig, S. 2004. Analysis of coffee, tea, cocoa, tobacco, spices, medicinal and aromatic plants, and related products. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 345-376.

Hatfield, R. D., H. G. Jung, G. Broderick, and T. C. Jenkins. 2007. Nutritional chemistry of forages. In: Forages: the science of grassland agriculture. Blackwell Publishing Professional, Ames, IA. p. 467-473.

Hoffman, R. M., R. C. Boston, D. Stefanovski, D. S. Kronfeld, and P. A. Harris. 2003. Obesity and diet affect glucose dynamics and insulin sensitivity in thoroughbred geldings. Journal of Animal Science. 81:2333-2342.

Hulme, A. C., and R. Narain. 1931. The ferricyanide method for the determination of reducing sugars. Biochemical Journal. 25:1051-1061.

Humphreys, M. O. 1989. Water-soluble carbohydrates in perennial ryegrass breeding. Grass and Forage Science. 44:231-244.

Jacobs, J., S. Rigby, F. McKenzie, M. Ryan, G. Ward, and S. Burch. 1989. Effect of nitrogen on pasture yield and quality for silage. In: Western Victoria proceedings of the Australian Agronomy Conference, Australian Society of Agronomy.

Jensen, K. B., P. Harrison, N. J. Chatterton, B. S. Bushman, and J. E. Creech. 2014. Seasonal trends in nonstructural carbohydrates in cool- and warm-season grasses. Crop Science. 54: 2328-2340.

Kagan, I. A., B. H. Kirch, C. D. Thatcher, J. R. Strickland, C. D. Teutsch, F. Elvinger, and R. S. Pleasant. 2011. Seasonal and diurnal variation in simple sugar and fructan composition of orchardgrass pasture and hay in the piedmont region of the united states. Journal of Equine Veterinary Science. 31:488-497.

Kane A. J., J. Traub-Dargatz, and W. C. Losinger. 2000. The occurrence and causes of lameness and laminitis in the U.S. horse population. In: Proceedings 46th Annual American Association of Equine Practitioners Convention, San Antonio, TX. p. 277–280.

Koolman, J., and K. H. Röhm. 1999. Atlas de poche de biochimie. Flammarion, France. p. 106-107.

Labhart, CH., J. Nösberger, and C. J. Nelson. 1983. Photosynthesis and degree of polymerization of fructan during reproductive growth of meadow fescue at two temperatures and two photon flux densities. Journal of Experimental Botany. 34:1037-1046.

Lacey, J. R., K. M. Olson-Rutz, M. R. Haferkamp, and G. A. Kennett. 1994. Effects of defoliation and competition on total nonstructural carbohydrates of spotted knapweed. Journal of Range Management. 47:481-484.

Lechtenburg, V. L., D. A. Holt, and H. W. Youngberg. 1972. Diurnal variation in nonstructural carbohydrates of *Festuca arundinacea* (Schreb.) with and without N fertilizer. Agronomy Journal. 64:302-305.

Longland, A. C., C. Barfoot, and P. A. Harris. 2016. Effects of grazing muzzles on intakes of dry matter and water-soluble carbohydrates by ponies grazing spring, summer, and autumn swards, as well as autumn swards of different heights. Journal of Equine Veterinary Science. 40:26-33.

Longland, A. C., and B. M. Byrd. 2006. Pasture nonstructural carbohydrates and equine laminitis. The Journal of Nutrition. 136:2099S-2102S.

Marten, G.C., F.E. Barton, and J.S. Shenk. 1989. Near infrared reflectance spectroscopy (NIRS): analysis of forage quality. Agriculture Handbook (USA), Washington, DC. p. 1-110.

Moore, K.J., and R. D. Hatfield. 1994. Carbohydrates and forage quality. In: Forage quality, evaluation, and utilization. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 229-280.

Murkin, J. M., and M. Argano. 2009. Near-infrared spectroscopy as an index of brain and tissue oxygenation. British Journal of Anaesthesia. 103:i3-i13.

Nilsson, U., R. Ost, M. Jagerstad, and D. Birkhed. 1988. Cereal fructans: in vitro and in vivo studies on availability in rats and humans. Journal of Nutrition. 118:1325-1330.

Olson, G.L., S. R. Smith, T.D. Phillips, G.D. Lacefield, and D.C. Ditsch. 2014a. PR-678 2014 Orchardgrass report. Agriculture Experiment Station, Lexington, KY.

Olson, G.L., S. R. Smith, T.D. Phillips, G.D. Lacefield, and D.C. Ditsch. 2014b. PR-679 2014 Tall fescue and bromegrass report. Agriculture Experiment Station, Lexington, KY.

Olson, G.L., S. R. Smith, T.D. Phillips, and G.D. Lacefield. 2014c. PR-680 2014 Timothy and Kentucky bluegrass report. Agriculture Experiment Station, Lexington, KY.

Olson, G.L., S. R. Smith, T.D. Phillips, and G.D. Lacefield. 2014d. PR-681 2014 Annual and perennial ryegrass and festulolium report. Agriculture Experiment Station, Lexington, KY.

Olson, G.L., S. R. Smith, G.D. Lacefield, T.D. Phillips, and L. M. Lawrence. 2014e. PR-685 2014 Cool-season grass horse grazing tolerance report. Agriculture Experiment Station, Lexington, KY.

Pavis, N., N. J. Chatterton, P. A. Harrison, S. Baumgartner, W. Praznik, J. Boucard, and M. P. Prud'homme. 2001. Structure of fructans in roots and leaf tissues of *Lolium perenne*. New Phytologist. 150:83-95.

Pavord, T., and M. Pavord. 2005. The complete equine veterinary manual: a comprehensive and instant guide to equine health. David and Charles, Newton Abbot, UK.

Pollitt, C. C. 2004. Equine laminitis. Clinical Techniques in Equine Practice. 3:34-44

Pollitt, C. C., M. Kyaw-Tanner, K. R. French, A. Van Eps, J. Hendrikz, and M. Daradka. 2003. Equine laminitis. In: Proceedings American Association of Equine Practitioners 49th Annual Convention. New Orleans, LA. 49:103-115.

Pollitt, C. C., and G. J. Milinovich. 2017. Experimental models of laminitis: oligofructose overload. In: Equine laminitis. John Wiley & Sons inc., p. 59-62.

Pollock, C. J., E. J. Lloyd, J. L. Stoddart, and H. Thomas. 1983. Growth, photosynthesis and assimilate partitioning in *Lolium temulentum* exposed to chilling temperatures. Physiologia Plantarum. 59: 257-262.

Pollock, C., and T. Jones. 1979. Seasonal patterns of fructan metabolism in forage grasses. New Phytologist. 83: 9-15.

Preiss, J., and C. Levi. 1980. Starch biosynthesis and degradation. In: P. K. Stumpf, and E. E. Conn, editors, The biochemistry of plants. Academic Press, p. 371-417.

Raven, P. H., R. F. Evert, and S. E. Eichhorn. 2005. Biology of plants. W. H. Freeman and Company, New York, NY. p. 15-19.

Redden, R. 2005. Laminitis: causes and cures. In: Advances in equine nutrition III. Nottingham University Press, Nottingham, UK. p. 439-444.

Ruiz, N. 2001. Near infrared spectroscopy: present and future applications. In: ASA Technical Bulletin. American Soybean Association, Singapore.

Sandra, E. K. 2004. Analysis of cereal food products. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 411-438.

Schwab, G. J., and M. W. Piersawl. 2010. AGR-200 Soil sampling and nutrient management in horse pastures. University of Kentucky College of Agriculture Cooperative Extension Services Bulletin, Lexington KY. p. 1-4.

Scotter, C. N. G., and S. J. Millar 2004. Analysis of baking products. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 439-463.

Sekiguchi, R., M. Ueno, and S. Kawano. 2004. Analysis of sugarcane. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 399-408.

Selim, S., K. Elo, S. Jaakkola, N. Karikoski, R. Boston, T. Reilas, S. Särkijärvi, M. Saastamoinen, and T. Kokkonen. 2015. Relationships among body condition, insulin

resistance and subcutaneous adipose tissue gene expression during the grazing season in mares. PLoS ONE. 10:e0125968.

Shenk, J.S., and Westerhaus, M.O., 1991. Populations structuring of near infrared spectra and modified partial least squares regression. Crop Science. 31:1548-1555.

Shenk, J. S., and M. O. Westerhaus. 1994. The application of near infared reflectance spectroscopy (NIRS) to forage analysis. In: Forage quality, evaluation, and utilization. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 406-449.

Shetty, N., R. Gislum, A.M. Dahl Jensen, and B. Boelt. 2012. Development of NIRS calibration models to assess year-to-year variation in total non-structural carbohydrates in grasses using PLSR. Chemometrics and Intelligent Laboratory Systems. 111: 34-38.

Shewmaker, G. E., H. F. Mayland, C. A. Roberts, P. A. Harrison, N. J. Chatterton, and D. A. Sleper. 2006. Daily carbohydrate accumulation in eight tall fescue cultivars. Grass and Forage Science. 61:413-421.

Slaughter, D. C., and J. A. Abbott. 2004. Analysis of fruits and vegetables. In: C. A. Roberts, J. Workman Jr., and J. B. Reeves III, editors, Near-infrared spectroscopy in agriculture. Agronomy Society of America, Crop Science Society of America, Soil Science Society of America, Madison, WI. p. 377-398.

Sprague, V. G., and J. T. Sullivan. 1950. Reserve carbohydrates in orchardgrass clipped periodically. Plant Physiology. 25:92-102.

Strangman, G., D. A. Boas, and J. P. Sutton. 2002. A non-invasive neuroimaging using near-infrared light. Biological Psychiatry. 52:679–93.

Taiz, L., and E. Zeiger. 1991. Plant Physiology. Sinauer Associated Inc. Publishers, Sunderland, MA. p. 219-248.

Van Eps, A. W., and C. C. Pollitt. 2006. Equine laminitis induced with oligofructose. Equine Veterinary Journal. 38:203–208.

Volaire, F., and F. Lelièvre. 1997. Production, persistence, and water-soluble carbohydrate accumulation in 21 contrasting populations of *Dactylis glomerata* L. subjected to severe drought in the south of France. Australian Journal of Agricultural Research. 48:933-944.

Walsh, D. M., and T. A. Burns. 2017. Historical perspective on equine laminitis. In: Equine Laminitis. John Wiley & Sons inc., p. 3-10.

Waite, R., and J. Boyd. 1953. The water-soluble carbohydrates of grasses. I.—Changes occurring during the normal life-cycle. Journal of the Science of Food and Agriculture 4:197-204.

Watts, K. A., and N. J. Chatterton. 2004. A review of factors affecting carbohydrate levels in forage. Journal of Equine Veterinary Science. 24:84-86.

Wilkins, P. W., and M. O. Humphreys. 2003. Progress in breeding perennial forage grasses in temperate agriculture. Journal of Agricultural Science. 140:129-150.

Wilkinson, J. M., J. D. Allen, R. Tunnicliffe, M. Smith, and P. C. Garnsworthy. 2014. Variation in composition of pre-grazed pasture herbage in the United Kingdom, 2006-2012. Animal Feed Science and Technology. 196:139-144.

Vita

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